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(54) Title: HUMAN PHOSPHATIDIC ACID PHOSPHATASE			
(57) Abstract This invention relates to a biotechnology invention concerning human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP- α (1 and 2), PAP- β and PAP- γ and uses thereof.			

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HUMAN PHOSPHATIDIC ACID PHOSPHATASE

Field of the Invention

This invention relates to human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP- α (1 and 2), PAP- β and PAP- γ and uses thereof. The invention encompasses biotechnology inventions, including biotechnology products and processes.

Background of the Invention

Phosphatidic acid phosphatase (PAP) (also referred to in the art as phosphatidate phosphohydrolase) is known to be an important enzyme for glycerolipid biosynthesis. In particular, PAP catalyzes the conversion of phosphatidic acid (PA) (also referred to in the art as phosphatidate) into diacylglycerol (DAG). DAG is an important branch point intermediate just downstream of PA in the pathways for biosynthesis of glycerophosphate-based phospholipids (Kent, Anal. Rev.Biochem. 64: 315-343, 1995).

In eukaryotic cells, PA, the precursor molecule for all glycerophospholipids, is converted either to CDP-diacylglycerol (CDP-DAG) by CDP-DAG synthase (CDS) or to DAG by phosphatidic acid phosphatase (PAP). In mammalian cells, CDP-DAG is the precursor to phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL); whereas diacylglycerol is the precursor to triacylglycerol (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) in all eukaryotic cells. Therefore, the partitioning of phosphatidic acid between

CDP-diacylglycerol and diacylglycerol is an important regulatory point in eukaryotic phospholipid metabolism (Shen et al., J. Biol. Chem. 271: 789-795, 1996).

5 In addition to being an important enzyme for glycerolipid biosynthesis, PAP is also an important enzyme for signal transduction. PAP catalyses the dephosphorylation of PA to DAG. DAG is a well-studied lipid second messenger which is essential for the activation of protein kinase C (Kent, Anal. Rev. Biochem. 64: 315-343, 1995); whereas PA itself is also a lipid messenger implicated in various signaling pathways such as NADPH oxidase activation and calcium mobilization (English, Cell Signal. 8: 341-347, 1996). The regulation of PAP activity can therefore affect the balance of divergent signaling processes that the cell receives in terms of PA and DAG (Brindley et al., Chem. Phys. Lipids 80: 45-57, 1996).

15 Various forms of PAP have been isolated in porcine (Kai et al., J. Biol. Chem. 271: 18931-18938, 1996) and rat species (Brindley et al., Chem. Phys. Lipids 80: 45-57, 1996). Furthermore, the putative amino acid sequence of murine PAP has been identified. Kai et al., *supra*. Prior to the instant invention, however, human PAP had not been identified or isolated.

25 Genes coding for PAP have been identified in *E. coli* (Dillon et al., J. Biol. Chem. 260: 12078-12083, 1985) and in mouse (Kai et al., J. Biol. Chem. 271: 18931-18938, 1996). Furthermore, the following GenBank human cDNA clones are available: accession nos. H17855, N75714, and W70040. No uses were known, however, for these polynucleotide sequences.

30

Accordingly, there is a need for the identification and isolation of human PAP and for methods of using human

PAP, for example, for the dephosphorylation of a substrate.

Summary of the Invention

5 It is therefore an object of the present invention to provide a polynucleotide sequences encoding three or more variants of human PAP, namely PAP- α (1 and 2), PAP- β and PAP- γ .

10 It is a further object to provide the isolated protein of these three variants.

It is yet a further object to provide a biotechnology method for preparing these variants via recombinant methods.

15 It is a further object to provide a biotechnology method of using these variants or human PA in general to synthesize DAG.

20 In accomplishing these and other objects there is provided an isolated polynucleotide encoding human phosphatidic acid phosphatase wherein the polynucleotide encodes a protein comprising a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 (SEQ ID NO:2) in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 (SEQ ID NO:4) in Figure 2, and
25 (iii) the sequence at amino acid number 1 to amino acid number 276 (SEQ ID NO:8) in Figure 4.

30 There is further provided an isolated human phosphatidic acid phosphatase protein, wherein the protein comprises a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 (SEQ ID NO:2) in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 (SEQ ID NO:4) in Figure 2, and (iii) the sequence at amino acid number 1 to amino acid number 276
35 (SEQ ID NO:8) in Figure 4.

There is further provided a method of preparing a human phosphatidic acid phosphatase- β protein comprising the steps of (i) transforming a host cell with an expression vector comprising a polynucleotide encoding human phosphatidic acid phosphatase, (ii) culturing the transformed host cells which express the protein and (iii) isolating the protein.

There is further provided a method of dephosphorylating a substrate comprising contacting the substrate with an effective amount of isolated human phosphatidic acid phosphatase protein such that the protein catalyzes the dephosphorylation of the substrate. It is further provided that the substrate of this method is selected from the group consisting of phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate. It is further provided that this method occurs *in vitro*, and comprises a step of isolating the dephosphorylated substrate. Additionally, the method can occur *in vivo*, and is effected by the administration of human phosphatidic acid phosphatase to a mammal in need thereof.

Brief Description of the Drawings

Figure 1 shows the DNA sequence of the cDNA insert of the human PAP- α 1 isolated herein and the corresponding amino acid sequence (SEQ ID NOS:1 and 2).

Figure 2 shows the DNA sequence of the cDNA insert of the human PAP- α 2 isolated herein and the corresponding amino acid sequence (SEQ ID NOS:3 and 4).

Figure 3 shows the DNA sequence of the cDNA insert of the human PAP- β isolated herein and the corresponding amino acid sequence (SEQ ID NOS:5 and 6).

Figure 4 shows the DNA sequence of the cDNA insert of the human PAP- γ isolated herein and the corresponding amino acid sequence (SEQ ID NOS:7 and 8).

Figure 5 shows amino acid sequences alignment of the murine PAP coding sequence and the coding sequences for human PAP- α (1 and 2), PAP- β and PAP- γ (SEQ ID NOS:9-13).

5 Figure 6 shows the effect of IL-1 β on PAP- β expression in human endothelial ECV304 cells using Northern blot analysis.

10 Figure 7 depicts a thin layer chromatography analysis demonstrating the increase in PA dephosphorylation in cells transfected with either the PAP- α 1 or PAP- α 2 cDNA expression plasmids.

Figure 8 shows the differential expression of PAP- α mRNA in various tumor versus normal tissues.

15 Figure 9 is a schematic representation of glycerophospholipid biosynthesis involving the conversion of PA to either DAG or CDP-DAG. The synthesis of PA to DAG involves the PAP enzyme, while the synthesis of PA to CPD-DAG involves the CDS enzyme.

Detailed Description of Preferred Embodiments

20 This invention relates to isolated human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP- α (1 and 2), PAP- β and PAP- γ .

25 Examples of the uses for human PAP include the following. PAP is an important tool for enzymatic catalysis of several biologically significant proteins. As discussed above, PAP catalyzes the dephosphorylation of PA to DAG. DAG, in turn, is essential for the activation of protein kinase C (Kent, Anal. Rev.Biochem. 64: 315-343, 1995).

30 Moreover, PAP catalyzes the dephosphorylation of lysophosphatidic acid (LPA), ceramide 1-phosphate (C-1-P), and sphingosine 1-phosphate (S-1-P) (Brindley et al., Chem. Phys. Lipids 80: 45-57, 1996). In the case of LPA, S-1-P, and C-1-P, the products of the PAP reaction are
35 monoacylglycerol, sphingosine, and ceramide,

respectively. PAP can control the balance of a wide spectrum of lipid mediators of cell activation and signal transduction by modulating the phosphorylated state of these lipids.

5 Additionally, the human PAP of the present invention are likely to define a new family of tumor suppressor genes that can be used as candidate genes for gene therapy for the treatment of certain tumors. The relationship of PAP and tumor suppression is evidenced
10 in findings that PAP activity is lower in fibroblast cell lines transformed with either the *ras* or *fps* oncogene than in the parental rat1 cell line (Brindley et al., *Chem. Phys. Lipids* 80: 45-57, 1996). Decrease in PAP activity in transformed cells correlates with a
15 concomitant increase in PA concentration. Moreover, elevated PAP activity and lower level of PA has been observed in contact-inhibited fibroblasts relative to proliferating and transformed fibroblasts (Brindley et al., *Chem. Phys. Lipids* 80: 45-57, 1996). Therefore, PAP
20 plays a role in decreasing cell division and as such can provide a useful tool in treating cancer.

 Additionally, PA, the substrate for the enzyme PAP, has been implicated in cytokine induced inflammatory responses (Bursten et al., *Circ. Shock* 44: 14-29, 1994;
25 Abraham et al., *J. Exp. Med.* 181: 569-575, 1995; Rice et al., *Proc. Natl. Acad. Sci. USA* 91: 3857-3861 1994; Leung et al., *Proc. Natl. Acad. Sci. USA* 92: 4813-4817, 1995) and the modulation of numerous protein kinases involved in signal transduction (English et al., *Chem. Phys. Lipids* 80: 117-132, 1996). Because of the possibility
30 that activation of human PAP expression can counter-balance the inflammatory response from cytokine stimulation through degradation of excess amount of PA in cells, the genes encoding human PAP can be used in gene
35 therapy for the treatment of inflammatory diseases.

Human PAP described herein can also be used in gene therapy for the treatment of obesity associated with diabetes. PAP activity is decreased in the livers and hearts of the grossly obese and insulin resistant JCR:LA corpulent rat compared to the control lean phenotype (Brindley et al., *Chem. Phys. Lipids* 80: 45-57, 1996). Human PAP described herein therefore can provide an important tool for the treatment of obesity associated with diabetes.

1. Human PAP

As used herein, "phosphatidic acid phosphatase" or "PAP" refers to a protein capable of catalyzing the dephosphorylation of PA to DAG. PAP also includes proteins capable of catalyzing the dephosphorylation of lysophosphatidic acid (LPA), ceramide 1-phosphate (C-1-P), and sphingosine 1-phosphate (S-1-P).

As used herein, "isolated" PAP denotes a degree of separation of the protein from other materials endogenous to the host organism. As used herein, "purified" denotes a higher degree of separation than isolated. A purified protein is sufficiently free of other materials endogenous to the host organism such that any remaining materials do not adversely affect the biological properties of the protein, for example, a purified protein is one sufficiently pure to be used in a pharmaceutical context.

As used herein, "human" PAP refers to PAP naturally occurring (or "native") in the human species, including natural variations due to allelic differences. The term "human PAP," however, is not limited to native human proteins, but also includes amino acid sequence variants of native human PAP that demonstrate PAP activity, as defined above.

Variants often exhibit the same qualitative biological activity as the naturally-occurring analogue,

although variants also are selected in order to modify the characteristics of PAP protein. In a preferred embodiment, therefore, human PAP includes the amino acid sequences of Figures 1-4 (SEQ ID NOS:2, 4, 6 and 8), being PAP- α 1, PAP- α 2, PAP- β and PAP- γ , respectively and variants thereof.

Amino acid sequence variants of the protein can be substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein which are not essential for biological activity. An example of a common deletion variant is a protein lacking transmembrane sequences. Another example is a protein lacking secretory signal sequences or signal sequences directing the protein to bind to a particular part of a cell.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and are designed to modulate one or more properties of the protein such as stability against proteolytic cleavage. Substitutions preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Of course, other amino acid substitutions can be undertaken.

Insertional variants contain fusion proteins such as those used to allow rapid purification of the protein and also can include hybrid proteins containing sequences from other proteins and polypeptides which are protein homologues.

Variants of human PAP also include fragments, analogs, derivatives, muteins and mimetics of the natural PAP protein that retain the ability to cause the beneficial results described above. Fragments of the human PAP protein refer to portions of the amino acid sequence of the PAP polypeptide that also retain this ability.

Variants can be generated directly from the human PAP protein itself by chemical modification by proteolytic enzyme digestion, or by combinations thereof. Additionally, methods of synthesizing polypeptides directly from amino acid residues also exist.

Non-peptide compounds that mimic the binding and function of the human PAP protein ("mimetics") can be produced by the approach outlined in Saragovi et al., *Science* 253: 792-95 (1991). Mimetics are peptide-containing molecules which mimic elements of protein secondary structure. See, for example, Johnson et al., "Peptide Turn Mimetics" in *BIOTECHNOLOGY AND PHARMACY*, Pezzuto et al., Eds., (Chapman and Hall, New York, 1993).

The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions. For the purposes of the present invention, appropriate mimetics can be considered to be the equivalent of the human PAP protein itself.

More typically, at least in the case of gene therapy, variants are created by recombinant techniques employing genomic or cDNA cloning methods. Site-specific

and region-directed mutagenesis techniques can be employed. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY vol. 1, ch. 8 (Ausubel et al. eds., J. Wiley & Sons 1989 & Supp. 1990-93); PROTEIN ENGINEERING (Oxender & Fox eds., A. Liss, Inc. 1987). In addition, linker-scanning and PCR-mediated techniques can be employed for mutagenesis. See PCR TECHNOLOGY (Erlich ed., Stockton Press 1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*. Protein sequencing, structure and modeling approaches for use with any of the above techniques are disclosed in PROTEIN ENGINEERING, *loc. cit.* and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*.

15 2. Polynucleotides Encoding Human PAP

The present invention further includes isolated polynucleotides encoding human phosphatidic acid phosphatase. As used herein, an "isolated" polynucleotide denotes a degree of separation of the polynucleotide from its naturally occurring environment, e.g., from its native intact genome. In a preferred embodiment, the isolated polynucleotides correspond to those shown in Figure 1 at nucleotide number 342 to nucleotide number 1193 of SEQ ID NO:1; Figure 2 at nucleotide number 342 to nucleotide number 1196 of SEQ ID NO:3; Figure 3 at nucleotide number 294 to nucleotide number 1226 of SEQ ID NO:5; and Figure 4 at nucleotide number 4 to nucleotide number 833 of SEQ ID NO:7.

30 The invention furthermore relates to a polynucleotide whose sequence is degenerate with respect to the sequences mentioned above in accordance with the nature of the genetic code. Degeneracy is often referred to as codon/anticodon wobble, and is discussed in Watson et al., MOLECULAR BIOLOGY OF THE GENE (4th ed. 1987) at 35 437-43.

The present invention further includes bases, nucleosides, nucleotides, oligonucleotides derived from the isolated polynucleotides of the present invention. The term "derived" when used in the context of the present invention connotes a degree of similarity that is sufficient to indicate the original polynucleotide from which hybrid forms, or portions thereof, were obtained. Also within the scope of the invention are so-called "polyamide" or "peptide" nucleic acids ("PNAs") derived from the polynucleotides of the present invention. PNAs are constructed by replacing the (deoxy)ribose phosphate backbone of a subject polynucleotide with an achiral polyamide backbone or the like. See Nielsen et al., *Science* 254: 1497-54 (1991).

The above polynucleotides and derivations thereof can be used as important tools in recombinant DNA and other protocols involving nucleic acid hybridization techniques. More specifically, oligonucleotides and nucleic acids derived from the isolated polynucleotides shown in Figures 1-4 (SEQ ID NOS:1, 3, 5, and 7) can be used as hybridization probes, capable of recognizing and specifically binding to complementary nucleic acid sequences, providing thereby a means of detecting, identifying, locating and measuring complementary nucleic acid sequences in a biological sample.

Biological samples include, among a great many others, blood or blood serum, lymph, ascites fluid, urine, microorganism or tissue culture medium, cell extracts, or the like, derived from a biological source, or a solution containing chemically synthesized protein, or an extract or solution prepared from such fluid from a biological source.

An oligonucleotide containing a modified nucleotide of the invention can be used as a primer to initiate nucleic acid synthesis at locations in a DNA or RNA molecule comprising the sequence complementary to the

oligonucleotide sequence. The synthesized nucleic acid strand would have incorporated, at its 5' terminus, the oligonucleotide primer bearing the invention and would, therefore, be detectable by exploitation of the characteristics of the detectable label. Two such primers, specific for different nucleotide sequences on complementary strands of dsDNA, can be used in the polymerase chain reaction (PCR) to synthesize and amplify the amount of a nucleotide sequence. The detectable label present on the primers will facilitate the identification of desired PCR products. PCR, combined with techniques for preparing complementary DNA (cDNA) can be used to amplify various RNAs, with oligonucleotide primers again serving both to provide points for initiation of synthesis in the cDNA duplex flanking the desired sequence and to identify the desired product. Primers labeled with the invention may also be utilized for enzymatic nucleic acid sequencing by the dideoxy chain-termination technique.

The invention can be applied to measure or quantitate the amount of DNA present in a sample. For instance, the concentration of nucleic acid can be measured by comparing detectable labels incorporated into the unknown nucleic acid with the concentration of detectable labels incorporated into known amounts of nucleic acid.

Such a comparative assessment can be done using biotin where the respective concentrations are determined by an enzyme-linked assay utilizing the streptavidin-alkaline phosphatase conjugate and a substrate yielding a soluble chromogenic or chemiluminescent signal.

3. Recombinant Production of Human PAP

In a further embodiment human PAP is expressed via recombinant methods known to those of skill in the art. The polynucleotides of the present invention can be

expressed in any number of different recombinant DNA expression systems to generate large amounts of protein, which can then be purified and used for the various applications of human PAP described above. Included within the present invention are proteins having native glycosylation sequences, and deglycosylated or unglycosylated proteins prepared by the methods described below.

Recombinant technology for producing desired proteins is known by ordinarily skilled artisans and includes providing a coding sequence for a desired protein, and operably linking the coding sequence to polynucleotide sequences capable of effecting its expression.

With regard to one aspect of the invention, it often is desirable to produce human PAP as a fusion protein, freed from upstream, downstream or intermediate sequences, or as a protein linked to leader sequences, effecting secretion of human PAP into cell culture medium.

A typical expression system will also contain control sequences necessary for transcription and translation of a message. Known control sequences include constitutive or inducible promoter systems, translational initiation signals (in eucaryotic expression), polyadenylation translation termination sites, and transcription terminating sequences. Expression vectors containing controls which permit operably linking of desired coding sequences to required control systems are known by the skilled artisan. Such vectors can be found which are operable in a variety of hosts.

Human PAP of the present invention may be produced in procaryotic cells using appropriate controls, such as *trp* or *lac* promoters, or in eucaryotic host cells, capable of effecting post-translational processing that

permits proteins to assume desired three-dimensional conformation. Eucaryotic control systems and expression vectors are known; including leu and glycolytic promoters useful in yeast, the viral SV40 and adenovirus and CMV promoters in mammalian cells, and the baculovirus system which is operable in insect cells. Plant vectors with suitable promoters, such as the nos promoter are also available.

Standard laboratory manuals (e.g., Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989) present standard techniques and methodologies for expressing polynucleotides encoding a desired protein, culturing appropriate cells, providing suitable expression conditions, and recovering a resulting protein from culture.

In preparing the inventive human PAP, a suitable polynucleotide encoding human PAP, constructed utilizing any of the foregoing techniques is operable linked to an expression vector which is then transformed into a compatible host. Host cells are cultured using conditions appropriate for growth. Expression of the desired human PAP is preferably induced after some predetermined growth level has occurred. Human PAP production is monitored and the desired protein isolated from culture either from a supernatant, or by first lysing host cells with an appropriate agent, or by other methods known to the skilled artisan.

In another preferred embodiment, a polynucleotide encoding human PAP is ligated into a mammalian expression vector. A preferred mammalian expression vector is the plasmid "pCE2." The plasmid pCE2 is derived from pREP7b (Leung, et al., Proc. Natl. Acad. Sci. USA, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1 α (EF-1 α) promoter and intron. The CMV enhancer of the pCE2 vector

is constructed from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' (SEQ ID NO:14) and 5'-CCTCACGCAT GCACCATGGT AATAGC-3' (SEQ ID NO:15). The EF-1 α promoter and intron (Uetsuki, et al., J. Biol. Chem., 264: 5791-5798, 1989) are constructed from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3' (SEQ ID NO:16) and 5'-GTAGTTTTCA CGGTACCTGA AATGGAAG-3' (SEQ ID NO:17). These 2 fragments are ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

In another preferred embodiment of the present invention, pCE2 containing a polynucleotide expressing human PAP is used to transform a host cell which then expresses the protein. Preferred host cells include the human embryonic kidney cell line 293-EBNA (Invitrogen, San Diego, CA), endothelial ECV304 cells, and epithelial A549 cells.

4. Dephosphorylation of Substrate

In another embodiment, the present invention includes a method of dephosphorylating a substrate by contacting the substrate with an effective amount of isolated human PAP. An "effective amount" of human PAP is an amount which will dephosphorylate a detectable amount of substrate. Such an amount can be determined empirically based on variables well known to those of skill in the art, such as reaction time and temperature.

In one embodiment, the substrate includes phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate. In another embodiment, the isolated human PAP includes PAP- α (1 and 2), PAP- β and PAP- γ and variants thereof.

In a further embodiment, the dephosphorylation of substrate occurs *in vitro*, by contacting a substrate with

recombinantly produced human PAP expressed by the methods described above. The dephosphorylated substrate is then isolated by standard isolation and purification methods, including for example, thin layer chromatography or high pressure liquid chromatography.

In another embodiment, the dephosphorylation of substrate occurs *in vivo* via the administration of human PAP to a mammal, preferably a human. "Administration" means delivery of human PAP protein to a mammal by methods known to those of skill in the art including, but not limited to: orally, for example in the form of pills, tablets, lacquer tablets, coated tablets, granules, hard gelatin capsules, soft gelatin capsules, solutions, syrups, emulsions, suspensions or aerosol mixtures; rectally, for example in the form of suppositories; parenterally, for example in the form of injection solutions or infusion solutions, microcapsules or rods; percutaneously, for example in the form of ointments or tinctures; transdermally; intravascularly, intracavitarily; intramuscularly; subcutaneously; and nasally, for example in the form of nasal sprays or inhalants.

The administration of human PAP protein includes the administration of the protein combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g. human serum albumin, are described for example in Remington's *Pharmaceutical Sciences* by E.W. Martin, which is hereby incorporated by reference. Such compositions will contain an effective amount of protein hereof together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host.

Such compositions should be stable for appropriate periods of time, preferably are acceptable for administration to humans and preferably are readily

manufacturable. Although pharmaceutical solution formulations are provided in liquid form appropriate for immediate use, formulations may also be provided in frozen or in lyophilized form. In the former case, the composition must be thawed prior to use. The latter form is often used to enhance the stability of the medicinal agent contained in the composition under a wide variety of storage conditions. Such lyophilized preparations are reconstituted prior to use by the addition of suitable pharmaceutically acceptable diluents, such as sterile water or sterile physiological saline solution.

Additionally, administration is meant to include delivery of human PAP protein to a mammal by means of gene therapy techniques, i.e., by the delivery of polynucleotides encoding human PAP to PAP-deficient cells, whereby human PAP is then expressed in the cell. Gene therapy techniques are known to those of skill in the art. For example, listing of present-day vectors suitable for use in gene therapy of the present invention is set forth in Hodgson, *Bio/Technology* 13: 222 (1995). See also, Culver et al., *Science*, 256:1550-62 (1992).

Additionally, liposome-mediated gene transfer is another suitable method for the introduction of a recombinant vector containing a polynucleotide encoding human PAP into a PAP-deficient cell. See Caplen et al., *Nature Med.* 1:39-46 (1995) and Zhu et al., *Science* 261:209-211 (1993).

Additionally, viral vector-mediated gene transfer is also a suitable method for the introduction of a recombinant vector containing the gene encoding human PAP into a PAP-deficient cell. Examples of appropriate viral vectors are adenovirus vectors. Detailed discussions of the use of adenoviral vectors for gene therapy can be found in Berkner, *Biotechniques* 6:616-629 (1988), Trapnell, *Advanced Drug Delivery Rev.* 12:185-199 (1993).

The following examples merely illustrate the invention and, as such, are not to be considered as limiting the invention set forth in the claims.

Example 1

Cloning and Expression of Human PAP- α , PAP- β and PAP- γ

Homology search of the Genbank database (Boguski, et al., Science 265:1993-1994, 1994) of expressed sequence tag (dbEST) using the murine PAP protein sequence (Kai et al., J. Biol. Chem. 271: 18931-18938, 1996) as probe identified several short stretches of human cDNA sequences with homology to the murine PAP protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA cloning projects carried out mainly by I.M.A.G.E. Consortium [LLNL] cDNA clones program. Based on the partial DNA sequences available in the GenBank database, the human cDNA clones that are homologous to the murine PAP protein sequence can be grouped into three classes, suggesting the presence of at least three different human PAP variants, designated as PAP- α , PAP- β , and PAP- γ here. For instance, a potential human PAP- α clone (GenBank #H17855) identified contains sequence homologous to aa 272-283 and the 3'-untranslated region of murine PAP; a potential human PAP- β clone (GenBank #W70040) identified contains sequence similarities corresponding to aa 175-251 of murine PAP; and a potential human PAP- γ clone (GenBank #N75714) identified contains sequences similarities corresponding to aa 18-142 of murine PAP. These cDNA clones were purchased (Genome Systems, St. Louis, MO) for further analysis. DNA sequence determination of the entire cDNA inserts of these clones showed clone H17855 contained sequences that are homologous to the N- and C-terminal sequences of murine PAP with a gap of about 150 bp that led to a frame shift in reading frame. This clone is most likely a

spuriously spliced form of PAP- α clone. Clone W70040 was found to be a full-length PAP- β clone, and clone N75714 was found to be a partial PAP- γ clone with an open reading frame homologous to the region from aa18 to the C-terminus of murine PAP.

To assemble a full-length functional PAP- α clone, synthetic oligonucleotides o_papalF, 5'-ggcatggtACCATGTTTGAC AAGACGCGGC-3' (SEQ ID NO:18), based on the N-terminal region of PAP- α and o_papalR, 5'-CATATGTAGTATTCAATGTA ACC-3' (SEQ ID NO:19), based on a region downstream of a Pst I site complementary to the coding strand of PAP- α were used to amplify the N-terminal coding region of PAP- α from a human lung cDNA library (Life Technologies, Inc., Gaithersburg, MD). The 450 bp Acc65 I - Pst I fragment generated was inserted into a Acc65 I / Pst I vector from pBluescript(II)SK(-) (Stratagene, San Diego, CA) for further analysis. DNA sequence analysis of the subclones obtained revealed at least two different classes of clones with sequences that diverged at the putative exon of interest, suggesting the presence of two alternatively spliced forms of PAP- α . These two alternatively spliced forms of PAP- α are designated as PAP- α 1 and PAP- α 2 here. Each of the individual 450 bp Acc65 I - Pst I fragment generated by PCR was combined with the 810 bp Pst I - Not I fragment derived from clone H17855 for ligation into a Acc65 I / Not I mammalian expression vector derived from pCE2 for the generation of expression plasmids for PAP- α 1 and PAP- α 2. The plasmid pCE2 was derived from pREP7b (Leung, et al., Proc. Natl. Acad. Sci. USA, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1 α (EF-1 α) promoter and intron. The CMV enhancer of the pCE2 vector was constructed from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' (SEQ ID NO:14) and 5'-

CCTCACGCAT GCACCATGGT AATAGC-3' (SEQ ID NO:15). The EF-1 α promoter and intron (Uetsuki, et al., J. Biol. Chem., 264: 5791-5798, 1989) was constructed from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3' (SEQ ID NO:16) and 5'-GTAGTTTTC ACGTACCTGA AATGGAAG-3' (SEQ ID NO:17). These 2 fragments were ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

The DNA sequence determined from clone N75714 was used as a probe to search for clones with overlapping sequences in the GenBank database. Clone Z43618 was found to contain an additional 5'-sequence with a potential ATG initiation codon. To assemble a full-length PAP- γ clone, synthetic oligonucleotides o_papglF, 5'-tgatggctag cATGCAGAGA AGATGGGTCT TCGTGCTGCT CGACGTG-3' (SEQ ID NO:20), based on the N-terminal region of PAP- γ and o_papglR, 5'-AGTGCGGGAT CCCATAAGTG GTTG-3', (SEQ ID NO:21) based on a region complementary to the coding strand of PAP- γ just downstream of its stop codon were used to generate the full-length coding region of PAP- γ by PCR using the clone N75714 as template. The 820 bp Nhe I - BamH I fragment obtained was then ligated into a Nhe I / BamH I mammalian expression vector derived from pCE2.

Figures 1, 2, 3 and 4 show the translated DNA sequences of the putative human cDNA clones for PAP- α 1, α 2, β and γ , (SEQ ID NOS:1, 3, 5 and 7) respectively. The designated ATG initiation site for translation of each cDNA clone fulfills the requirement for an adequate initiation site according to Kozak (Kozak, Critical Rev. Biochem. Mol. Biol. 27:385-402, 1992).

The amino acid sequence of each open reading frame (Figures 1, 2, 3 and 4 (SEQ ID NOS:2, 4, 6 and 8)) was used as the query sequence to search for homologous sequences in protein databases. Search of the Genbank

database from the National Center for Biotechnology Information (NCBI) using the blastp program showed that these proteins are most homologous to the murine PAP sequence (Kai et al., J. Biol. Chem. 271: 18931-18938, 1996), and a rat endoplasmic reticulum resident transmembrane protein of unknown function, Dri 42, whose expression is up-regulated during epithelial differentiation (Barila et al., J. Biol. Chem. 271: 29928-29936, 1996).

Example 2

Activation of PAP- β Transcription by IL1- β

It is possible that activation of PAP- β expression can counter-balance the inflammatory response from IL-1 β stimulation through degradation of the excess amount of PA in cells. To determine whether IL1- β , an inflammatory cytokine, would activate the transcription of PAP mRNAs, Northern analysis of PAP- β mRNA levels (Fig. 6) was performed in human endothelial ECV304 cells at various times after IL-1 β stimulation. Figure 6 shows that PAP- β mRNA expression was induced after incubation of ECV304 cells with IL-1 β after at least 6 hours, suggesting that PAP- β is a late-response gene to IL-1 β stimulation. This indicates that human PAP may act to reduce IL-1 β induced inflammation by degrading excess PA in cells.

Example 3

PAP- α 1 and PAP- α 2 Dephosphorylation of PA to DAG

The expression of PAP- α 1 and PAP- α 2 cDNA was found to increase PA dephosphorylation in mammalian cells. The expression plasmids for PAP- α 1, PAP- α 2 and the control vector were transiently transfected into 293-EBNA (EB293) cells (Invitrogen, San Diego, CA) using the lipofectant DOTAP (Boehringer Mannheim, Indianapolis, IN). PAP activities were followed by TLC analysis based on the conversion of [C^{14}]PA (DuPont NEN, Boston, MA) to

[C¹⁴]DAG using membrane fractions isolated from the various cell extracts. Figure 7 shows membrane fractions derived from cells transfected with either the PAP- α 1 (lanes 6 and 7) or PAP- α 2 (lanes 8 and 9) produced more [C¹⁴]DAG those from untransfected cells (lanes 2 and 3) or from cells transfected with the control pCE2 vector (lanes 4 and 5). In this particular chromatography system, DAG can be resolved into two bands, possibly due to heterogeneity in the acyl-chains. It appears that PAP- α 1 and PAP- α 2 preferentially dephosphorylate different species of PA as evidenced by the change in relative intensity of the two DAG bands (lanes 6 to 9).

Example 4
Differential Expression of PAP- α mRNA in
Selected Tumor Versus Normal Tissues

The possibility that PAP- α expression can degrade the excess amount of PA in cells suggests that PAP- α may be down-regulated in tumor cells when compared to normal cells, as tumor cells tend to be more inflammatory due to a possibly higher level of PA when compared to normal or resting cells. To test this hypothesis, Northern analysis using PAP- α (1 and 2) cDNA probe was performed on RNA blots derived from various matching pairs of tumor and normal tissues (Invitrogen, Carlsbad, CA). Figure 8 shows the expression levels of PAP- α mRNA are substantially higher in five out of eight of the normal tissues examined; namely, colon, rectal, breast, fallopian tube, and ovarian tissues when compared to the corresponding tumor tissues.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: LEUNG, David W.
TOMPKINS, Christopher K.
- (ii) TITLE OF INVENTION: HUMAN PHOSPHATIDIC ACID PHOSPHATASE
- (iii) NUMBER OF SEQUENCES: 21
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Foley & Lardner
 - (B) STREET: 3000 K Street, N.W., Suite 500
 - (C) CITY: Washington
 - (D) STATE: D.C.
 - (E) COUNTRY: USA
 - (F) ZIP: 20007-5109
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/842,827
 - (B) FILING DATE: 17-APR-1997
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: BENT, Stephen A.
 - (B) REGISTRATION NUMBER: 29,768
 - (C) REFERENCE/DOCKET NUMBER: 77319/125
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (202)672-5300
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 - (C) TELEX: 904136

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1563 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 342..1193

- (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) LOCATION: 342..1193

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGTGGGAG AGAGCGCCGG GATCCGGACG GGGTAGCAAC CGGGGCAGGC CGTGCCGGCT	60
GAGGAGGTCC TGAGGCTACA GAGCTGCCGC GGCTGGCACA CGAGCGCCTC GGCAC TAACC	120

GAGTGTTCGC GGGGGCTGTG AGGGGAGGGC CCCGGGCGCC ATTGCTGGCG GTGGGAGCGC	180
CGCCCCGGTCT CAGCCCCGCC TCGGCTGCTC TCCTCCTCCG GCTGGGAGGG GCCGTATCTC	240
GGGGCCGTCG CCAGCCCCGG CCCGGGCTCG ATAATCAAGG GCCTCGGCCG TCGTCCCGCA	300
CCTCATTCCA TCGCCCTTGC CGGGCAGCCC GGGCAGAGAC C ATG TTT GAC AAG	353
Met Phe Asp Lys	
1	
ACG CGG CTG CCG TAC GTG GCC CTC GAT GTG CTC TGC GTG TTG CTG GCT	401
Thr Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys Val Leu Leu Ala	
5 10 15 20	
GGA TTG CCT TTT GCA ATT CTT ACT TCA AGG CAT ACC CCC TTC CAA CGA	449
Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr Pro Phe Gln Arg	
25 30 35	
GGA GTA TTC TGT AAT GAT GAG TCC ATC AAG TAC CCT TAC AAA GAA GAC	497
Gly Val Phe Cys Asn Asp Glu Ser Ile Lys Tyr Pro Tyr Lys Glu Asp	
40 45 50	
ACC ATA CCT TAT GCG TTA TTA GGT GGA ATA ATC ATT CCA TTC AGT ATT	545
Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Ile Ile Pro Phe Ser Ile	
55 60 65	
ATC GTT ATT ATT CTT GGA GAA ACC CTG TCT GTT TAC TGT AAC CTT TTG	593
Ile Val Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr Cys Asn Leu Leu	
70 75 80	
CAC TCA AAT TCC TTT ATC AGG AAT AAC TAC ATA GCC ACT ATT TAC AAA	641
His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile Ala Thr Ile Tyr Lys	
85 90 95 100	
GCC ATT GGA ACC TTT TTA TTT GGT GCA GCT GCT AGT CAG TCC CTG ACT	689
Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala Ala Ser Gln Ser Leu Thr	
105 110 115	
GAC ATT GCC AAG TAT TCA ATA GGC AGA CTG CGG CCT CAC TTC TTG GAT	737
Asp Ile Ala Lys Tyr Ser Ile Gly Arg Leu Arg Pro His Phe Leu Asp	
120 125 130	
GTT TGT GAT CCA GAT TGG TCA AAA ATC AAC TGC AGC GAT GGT TAC ATT	785
Val Cys Asp Pro Asp Trp Ser Lys Ile Asn Cys Ser Asp Gly Tyr Ile	
135 140 145	
GAA TAC TAC ATA TGT CGA GGG AAT GCA GAA AGA GTT AAG GAA GGC AGG	833
Glu Tyr Tyr Ile Cys Arg Gly Asn Ala Glu Arg Val Lys Glu Gly Arg	
150 155 160	
TTG TCC TTC TAT TCA GGC CAC TCT TCG TTT TCC ATG TAC TGC ATG CTG	881
Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser Met Tyr Cys Met Leu	
165 170 175 180	
TTT GTG GCA CTT TAT CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA AGA	929
Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys Gly Asp Trp Ala Arg	
185 190 195	
CTC TTA CGC CCC ACA CTG CAA TTT GGT CTT GTT GCC GTA TCC ATT TAT	977
Leu Leu Arg Pro Thr Leu Gln Phe Gly Leu Val Ala Val Ser Ile Tyr	
200 205 210	
GTG GGC CTT TCT CGA GTT TCT GAT TAT AAA CAC CAC TGG AGC GAT GTG	1025
Val Gly Leu Ser Arg Val Ser Asp Tyr Lys His His Trp Ser Asp Val	
215 220 225	

TTG ACT GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT GTA 1073
 Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala Val
 230 235 240

TAT GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA AAA 1121
 Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg Lys
 245 250 255 260

GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT GGG AAT 1169
 Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr Gly Asn
 265 270 275

CAC TAT CCG AGC AAT CAC CAG CCT TGAAAGGCAG CAGGGTGCCC AGGTGAAGCT 1223
 His Tyr Pro Ser Asn His Gln Pro
 280

GGCCTGTTTT CTAAAGGAAA ATGATTGCCA CAAGGCAAGA GGATGCATCT TTCTTCCTGG 1283

TGTACAAGCC TTAAAGACT TCTGCTGCTG ATATGCCTCT TGGATGCACA CTTTGTGTGT 1343

ACATAGTTAC CTTTAACTCA GTGGTTATCT AATAGCTCTA AACTCATTAA AAAA ACTCCA 1403

AGCCTTCCAC CAAAACAGTG CCCACCTGT ATACATTTTTT ATTAAAAAAA TGTAATGCTT 1463

ATGTATAAAC ATGTATGTAA TATGCTTTCT ATGAATGATG TTTGATTTAA ATATAATACA 1523

TATTAAAATG TATGGGAGAA CCAAAAAAAA AAAAAAAA 1563

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 284 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Phe Asp Lys Thr Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys
 1 5 10 15

Val Leu Leu Ala Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr
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Pro Phe Gln Arg Gly Val Phe Cys Asn Asp Glu Ser Ile Lys Tyr Pro
 35 40 45

Tyr Lys Glu Asp Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Ile Ile
 50 55 60

Pro Phe Ser Ile Ile Val Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr
 65 70 75 80

Cys Asn Leu Leu His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile Ala
 85 90 95

Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala Ser
 100 105 110

Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg Leu Arg Pro
 115 120 125

His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser Lys Ile Asn Cys Ser
 130 135 140

Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg Gly Asn Ala Glu Arg Val
 145 150 155 160
 Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser Met
 165 170 175
 Tyr Cys Met Leu Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys Gly
 180 185 190
 Asp Trp Ala Arg Leu Leu Arg Pro Thr Leu Gln Phe Gly Leu Val Ala
 195 200 205
 Val Ser Ile Tyr Val Gly Leu Ser Arg Val Ser Asp Tyr Lys His His
 210 215 220
 Trp Ser Asp Val Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile
 225 230 235 240
 Leu Val Ala Val Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe
 245 250 255
 Lys Glu Arg Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro
 260 265 270
 Thr Thr Gly Asn His Tyr Pro Ser Asn His Gln Pro
 275 280

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1566 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ix) FEATURE:
- (A) NAME/KEY: CDS
 - (B) LOCATION: 342..1196

- (ix) FEATURE:
- (A) NAME/KEY: mat_peptide
 - (B) LOCATION: 342..1196

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTGTGGGAG AGAGCGCCGG GATCCGGACG GGGTAGCAAC CGGGGCAGGC CGTGCCGGCT 60
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 CGCCCGGTCT CAGCCCGCCC TCGGCTGCTC TCCTCCTCCG GCTGGGAGGG GCCGTATCTC 240
 GGGGCCGTCG CCAGCCCCGG CCCGGGCTCG ATAATCAAGG GCCTCGGCCG TCGTCCCGCA 300
 CCTCATTCCA TCGCCCTTGC CGGGCAGCCC GGGCAGAGAC C ATG TTT GAC AAG 353
 Met Phe Asp Lys
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AGT ACC GCC GCA TCC ACT GTC CTC ATC CTA GTG GGG GTT GGC TTG CCC Ser Thr Ala Ala Ser Thr Val Leu Ile Leu Val Gly Val Gly Leu Pro 55 60 65	545
GTT TCC TCT ATT ATT CTT GGA GAA ACC CTG TCT GTT TAC TGT AAC CTT Val Ser Ser Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr Cys Asn Leu 70 75 80	593
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AAA GCC ATT GGA ACC TTT TTA TTT GGT GCA GCT GCT AGT CAG TCC CTG Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala Ala Ser Gln Ser Leu 105 110 115	689
ACT GAC ATT GCC AAG TAT TCA ATA GGC AGA CTG CGG CCT CAC TTC TTG Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg Leu Arg Pro His Phe Leu 120 125 130	737
GAT GTT TGT GAT CCA GAT TGG TCA AAA ATC AAC TGC AGC GAT GGT TAC Asp Val Cys Asp Pro Asp Trp Ser Lys Ile Asn Cys Ser Asp Gly Tyr 135 140 145	785
ATT GAA TAC TAC ATA TGT CGA GGG AAT GCA GAA AGA GTT AAG GAA GGC Ile Glu Tyr Tyr Ile Cys Arg Gly Asn Ala Glu Arg Val Lys Glu Gly 150 155 160	833
AGG TTG TCC TTC TAT TCA GGC CAC TCT TCG TTT TCC ATG TAC TGC ATG Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser Met Tyr Cys Met 165 170 175 180	881
CTG TTT GTG GCA CTT TAT CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA Leu Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys Gly Asp Trp Ala 185 190 195	929
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GTG TTG ACT GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT Val Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala 230 235 240	1073
GTA TAT GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA Val Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg 245 250 255 260	1121
AAA GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT GGG Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr Gly 265 270 275	1169
AAT CAC TAT CCG AGC AAT CAC CAG CCT TGAAAGGCAG CAGGGTGCCCC Asn His Tyr Pro Ser Asn His Gln Pro 280 285	1216

AGGTGAAGCT GGCCTGTTTT CTAAAGGAAA ATGATTGCCA CAAGGCAAGA GGATGCATCT	1276
TTCTTCCTGG TGTACAAGCC TTAAAGACT TCTGCTGCTG ATATGCCTCT TGGATGCACA	1336
CTTTGTGTGT ACATAGTTAC CTTTAACTCA GTGGTTATCT AATAGCTCTA AACTCATTA	1396
AAAAACTCCA AGCCTTCCAC CAAAACAGTG CCCCACCTGT ATACATTTTT ATTAAAAAA	1456
TGTAATGCTT ATGTATAAAC ATGTATGTAA TATGCTTTCT ATGAATGATG TTTGATTAA	1516
ATATAATACA TATTAAAATG TATGGGAGAA CCAAAAAAA AAAAAAAA	1566

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 285 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Phe	Asp	Lys	Thr	Arg	Leu	Pro	Tyr	Val	Ala	Leu	Asp	Val	Leu	Cys	1	5	10	15
Val	Leu	Leu	Ala	Ser	Met	Pro	Met	Ala	Val	Leu	Lys	Leu	Gly	Gln	Ile	20	25	30	
Tyr	Pro	Phe	Gln	Arg	Gly	Phe	Phe	Cys	Lys	Asp	Asn	Ser	Ile	Asn	Tyr	35	40	45	
Pro	Tyr	His	Asp	Ser	Thr	Ala	Ala	Ser	Thr	Val	Leu	Ile	Leu	Val	Gly	50	55	60	
Val	Gly	Leu	Pro	Val	Ser	Ser	Ile	Ile	Leu	Gly	Glu	Thr	Leu	Ser	Val	65	70	75	80
Tyr	Cys	Asn	Leu	Leu	His	Ser	Asn	Ser	Phe	Ile	Ser	Asn	Asn	Tyr	Ile	85	90	95	
Ala	Thr	Ile	Tyr	Lys	Ala	Ile	Gly	Thr	Phe	Leu	Phe	Gly	Ala	Ala	Ala	100	105	110	
Ser	Gln	Ser	Leu	Thr	Asp	Ile	Ala	Lys	Tyr	Ser	Ile	Gly	Arg	Leu	Arg	115	120	125	
Pro	His	Phe	Leu	Asp	Val	Cys	Asp	Pro	Asp	Trp	Ser	Lys	Ile	Asn	Cys	130	135	140	
Ser	Asp	Gly	Tyr	Ile	Glu	Tyr	Tyr	Ile	Cys	Arg	Gly	Asn	Ala	Glu	Arg	145	150	155	160
Val	Lys	Glu	Gly	Arg	Leu	Ser	Phe	Tyr	Ser	Gly	His	Ser	Ser	Phe	Ser	165	170	175	
Met	Tyr	Cys	Met	Leu	Phe	Val	Ala	Leu	Tyr	Leu	Gln	Ala	Arg	Met	Lys	180	185	190	
Gly	Asp	Trp	Ala	Arg	Leu	Leu	Arg	Pro	Thr	Leu	Gln	Phe	Gly	Leu	Val	195	200	205	
Ala	Val	Ser	Ile	Tyr	Val	Gly	Leu	Ser	Arg	Val	Ser	Asp	Tyr	Lys	His	210	215	220	

His Trp Ser Asp Val Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala
 225 230 235 240
 Ile Leu Val Ala Val Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser
 245 250 255
 Phe Lys Glu Arg Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr
 260 265 270
 Pro Thr Thr Gly Asn His Tyr Pro Ser Asn His Gln Pro
 275 280 285

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1362 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 294..1226

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 294..1226

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGCGCAGCTC TGCAAAAGTT TCTGCTCGGG ATCTGGCTCT CTTCCCCTTG GACTTTAGAA 60
 CGATTTAGGG TTGACAGAGG AAAGCAGAGG CGCGCAGGAG GAGCAGAAAA CACCACCTTC 120
 TGCAGTTGGA GGCAGGCAGC CCCGGCTGCA CTCTAGCCGC CGCGCCCGGA GCCGGGGCCG 180
 ACCCGCCACT ATCCGCAGCA GCCTCGGCCA GGAGGCGACC CGGGCGCCTG GGTGTGTGGC 240
 TGCTGTTGCG GGACGTCTTC GCGGGGCGGG AGGCTCGCGC CGCAGCCAGC GCC ATG 296
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 1
 CAA AAC TAC AAG TAC GAC AAA GCG ATC GTC CCG GAG AGC AAG AAC GGC 344
 Gln Asn Tyr Lys Tyr Asp Lys Ala Ile Val Pro Glu Ser Lys Asn Gly
 5 10 15
 GGC AGC CCG GCG CTC AAC AAC AAC CCG AGG AGG AGC GGC AGC AAG CGG 392
 Gly Ser Pro Ala Leu Asn Asn Asn Pro Arg Arg Ser Gly Ser Lys Arg
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 GTG CTG CTC ATC TGC CTC GAC CTC TTC TGC CTC TTC ATG GCG GGC CTC 440
 Val Leu Leu Ile Cys Leu Asp Leu Phe Cys Leu Phe Met Ala Gly Leu
 35 40 45
 CCC TTC CTC ATC ATC GAG ACA AGC ACC ATC AAG CCT TAC CAC CGA GGG 488
 Pro Phe Leu Ile Ile Glu Thr Ser Thr Ile Lys Pro Tyr His Arg Gly
 50 55 60 65
 TTT TAC TGC AAT GAT GAG AGC ATC AAG TAC CCA CTG AAA ACT GGT GAG 536
 Phe Tyr Cys Asn Asp Glu Ser Ile Lys Tyr Pro Leu Lys Thr Gly Glu
 70 75 80

ACA ATA AAT GAC GCT GTG CTC TGT GCC GTG GGG ATC GTC ATT GCC ATC Thr Ile Asn Asp Ala Val Leu Cys Ala Val Gly Ile Val Ile Ala Ile 85 90 95	584
CTC GCG ATC ATC ACG GGG GAA TTC TAC CGG ATC TAT TAC CTG AAG AAG Leu Ala Ile Ile Thr Gly Glu Phe Tyr Arg Ile Tyr Tyr Leu Lys Lys 100 105 110	632
TCG CGG TCG ACG ATT CAG AAC CCC TAC GTG GCA GCA CTC TAT AAG CAA Ser Arg Ser Thr Ile Gln Asn Pro Tyr Val Ala Ala Leu Tyr Lys Gln 115 120 125	680
GTG GGC TGC TTC CTC TTT GGC TGT GCC ATC AGC CAG TCT TTC ACA GAC Val Gly Cys Phe Leu Phe Gly Cys Ala Ile Ser Gln Ser Phe Thr Asp 130 135 140 145	728
ATT GCC AAA GTG TCC ATA GGG CGC CTG CGT CCT CAC TTC TTG AGT GTC Ile Ala Lys Val Ser Ile Gly Arg Leu Arg Pro His Phe Leu Ser Val 150 155 160	776
TGC AAC CCT GAT TTC AGC CAG ATC AAC TGC TCT GAA GGC TAC ATT CAG Cys Asn Pro Asp Phe Ser Gln Ile Asn Cys Ser Glu Gly Tyr Ile Gln 165 170 175	824
AAC TAC AGA TGC AGA GGT GAT GAC AGC AAA GTC CAG GAA GCC AGG AAG Asn Tyr Arg Cys Arg Gly Asp Asp Ser Lys Val Gln Glu Ala Arg Lys 180 185 190	872
TCC TTC TTC TCT GGC CAT GCC TCC TTC TCC ATG TAC ACT ATG CTG TAT Ser Phe Phe Ser Gly His Ala Ser Phe Ser Met Tyr Thr Met Leu Tyr 195 200 205	920
TTG GTG CTA TAC CTG CAG GCC CGC TTC ACT TGG CGA GGA GCC CGC CTG Leu Val Leu Tyr Leu Gln Ala Arg Phe Thr Trp Arg Gly Ala Arg Leu 210 215 220 225	968
CTC CGG CCC CTC CTG CAG TTC ACC TTG ATC ATG ATG GCC TTC TAC ACG Leu Arg Pro Leu Leu Gln Phe Thr Leu Ile Met Met Ala Phe Tyr Thr 230 235 240	1016
GGA CTG TCT CGC GTA TCA GAC CAC AAG CAC CAT CCC AGT GAT GTT CTG Gly Leu Ser Arg Val Ser Asp His Lys His His Pro Ser Asp Val Leu 245 250 255	1064
GCA GGA TTT GCT CAA GGA GCC CTG GTG GCC TGC TGC ATA GTT TTC TTC Ala Gly Phe Ala Gln Gly Ala Leu Val Ala Cys Cys Ile Val Phe Phe 260 265 270	1112
GTG TCT GAC CTC TTC AAG ACT AAG ACG ACG CTC TCC CTG CCT GCC CCT Val Ser Asp Leu Phe Lys Thr Lys Thr Thr Leu Ser Leu Pro Ala Pro 275 280 285	1160
GCT ATC CGG AAG GAA ATC CTT TCA CCT GTG GAC ATT ATT GAC AGG AAC Ala Ile Arg Lys Glu Ile Leu Ser Pro Val Asp Ile Ile Asp Arg Asn 290 295 300 305	1208
AAT CAC CAC AAC ATG ATG TAGGTGCCAC CCACCTCCTG AGCTGTTTTT Asn His His Asn Met Met 310	1256
GTAAATGAC TGCTGACAGC AAGTTCTTGC TGCTCTCCAA TCTCATCAGA CAGTAGAATG	1316
TAGGGAAAAA CTTTTGCCCG ACTGATTTTTT AAAAAAAAAA AAAAAA	1362

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 311 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Gln Asn Tyr Lys Tyr Asp Lys Ala Ile Val Pro Glu Ser Lys Asn
 1             5             10             15
Gly Gly Ser Pro Ala Leu Asn Asn Asn Pro Arg Arg Ser Gly Ser Lys
                20             25             30
Arg Val Leu Leu Ile Cys Leu Asp Leu Phe Cys Leu Phe Met Ala Gly
 35             40             45
Leu Pro Phe Leu Ile Ile Glu Thr Ser Thr Ile Lys Pro Tyr His Arg
 50             55             60
Gly Phe Tyr Cys Asn Asp Glu Ser Ile Lys Tyr Pro Leu Lys Thr Gly
 65             70             75             80
Glu Thr Ile Asn Asp Ala Val Leu Cys Ala Val Gly Ile Val Ile Ala
                85             90             95
Ile Leu Ala Ile Ile Thr Gly Glu Phe Tyr Arg Ile Tyr Tyr Leu Lys
100             105             110
Lys Ser Arg Ser Thr Ile Gln Asn Pro Tyr Val Ala Ala Leu Tyr Lys
115             120             125
Gln Val Gly Cys Phe Leu Phe Gly Cys Ala Ile Ser Gln Ser Phe Thr
130             135             140
Asp Ile Ala Lys Val Ser Ile Gly Arg Leu Arg Pro His Phe Leu Ser
145             150             155             160
Val Cys Asn Pro Asp Phe Ser Gln Ile Asn Cys Ser Glu Gly Tyr Ile
                165             170             175
Gln Asn Tyr Arg Cys Arg Gly Asp Asp Ser Lys Val Gln Glu Ala Arg
180             185             190
Lys Ser Phe Phe Ser Gly His Ala Ser Phe Ser Met Tyr Thr Met Leu
195             200             205
Tyr Leu Val Leu Tyr Leu Gln Ala Arg Phe Thr Trp Arg Gly Ala Arg
210             215             220
Leu Leu Arg Pro Leu Leu Gln Phe Thr Leu Ile Met Met Ala Phe Tyr
225             230             235             240
Thr Gly Leu Ser Arg Val Ser Asp His Lys His His Pro Ser Asp Val
                245             250             255
Leu Ala Gly Phe Ala Gln Gly Ala Leu Val Ala Cys Cys Ile Val Phe
260             265             270
Phe Val Ser Asp Leu Phe Lys Thr Lys Thr Thr Leu Ser Leu Pro Ala
275             280             285
Pro Ala Ile Arg Lys Glu Ile Leu Ser Pro Val Asp Ile Ile Asp Arg
290             295             300

```

Asn Asn His His Asn Met Met
305 310

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1232 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 4..833

- (ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 4..833

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACC ATG CAG CGG AGG TGG GTC TTC GTG CTG CTC GAC GTG CTG TGC TTA	48
Met Gln Arg Arg Trp Val Phe Val Leu Leu Asp Val Leu Cys Leu	
1 5 10 15	
CTG GTC GCC TCC CTG CCC TTC GCT ATC CTG ACG CTG GTG AAC GCC CCG	96
Leu Val Ala Ser Leu Pro Phe Ala Ile Leu Thr Leu Val Asn Ala Pro	
20 25 30	
TAC AAG CGA GGA TTT TAC TGC GGG GAT GAC TCC ATC CGG TAC CCC TAC	144
Tyr Lys Arg Gly Phe Tyr Cys Gly Asp Asp Ser Ile Arg Tyr Pro Tyr	
35 40 45	
CGT CCA GAT ACC ATC ACC CAC GGG CTC ATG GCT GGG GTC ACC ATC ACG	192
Arg Pro Asp Thr Ile Thr His Gly Leu Met Ala Gly Val Thr Ile Thr	
50 55 60	
GCC ACC GTC ATC CTT GTC TCG GCC GGG GAA GCC TAC CTG GTG TAC ACA	240
Ala Thr Val Ile Leu Val Ser Ala Gly Glu Ala Tyr Leu Val Tyr Thr	
65 70 75	
GAC CGG CTC TAT TCT CGC TCG GAC TTC AAC AAC TAC GTG GCT GCT GTA	288
Asp Arg Leu Tyr Ser Arg Ser Asp Phe Asn Asn Tyr Val Ala Ala Val	
80 85 90 95	
TAC AAG GTG CTG GGG ACC TTC CTG TTT GGG GCT GCC GTG AGC CAG TCT	336
Tyr Lys Val Leu Gly Thr Phe Leu Phe Gly Ala Ala Val Ser Gln Ser	
100 105 110	
CTG ACA GAC CTG GCC AAG TAC ATG ATT GGG CGT CTG AAG CCC AAC TTC	384
Leu Thr Asp Leu Ala Lys Tyr Met Ile Gly Arg Leu Lys Pro Asn Phe	
115 120 125	
CTA GCC GTC TGC GAC CCC GAC TGG AGC CGG GTC AAC TGC TCG GTC TAT	432
Leu Ala Val Cys Asp Pro Asp Trp Ser Arg Val Asn Cys Ser Val Tyr	
130 135 140	
GTG CAG CTG GAG AAG GTG TGC AGG GGA AAC CCT GCT GAT GTC ACC GAG	480
Val Gln Leu Glu Lys Val Cys Arg Gly Asn Pro Ala Asp Val Thr Glu	
145 150 155	
GCC AGG TTG TCT TTC TAC TCG GGA CAC TCT TCC TTT GGG ATG TAC TGC	528
Ala Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Gly Met Tyr Cys	
160 165 170 175	

ATG GTG TTC TTG GCG CTG TAT GTG CAG GCA CGA CTC TGT TGG AAG TGG 576
 Met Val Phe Leu Ala Leu Tyr Val Gln Ala Arg Leu Cys Trp Lys Trp
 180 185 190

GCA CGG CTG CTG CGA CCC ACA GTC CAG TTC TTC CTG GTG GCC TTT GCC 624
 Ala Arg Leu Leu Arg Pro Thr Val Gln Phe Phe Leu Val Ala Phe Ala
 195 200 205

CTC TAC GTG GGC TAC ACC CGC GTG TCT GAT TAC AAA CAC CAC TGG AGC 672
 Leu Tyr Val Gly Tyr Thr Arg Val Ser Asp Tyr Lys His His Trp Ser
 210 215 220

GAT GTC CTT GTT GGC CTC CTG CAG GGG GCA CTG GTG GCT GCC CTC ACT 720
 Asp Val Leu Val Gly Leu Leu Gln Gly Ala Leu Val Ala Ala Leu Thr
 225 230 235

GTC TGC TAC ATC TCA GAC TTC TTC AAA GCC CGA CCC CCA CAG CAC TGT 768
 Val Cys Tyr Ile Ser Asp Phe Phe Lys Ala Arg Pro Pro Gln His Cys
 240 245 250 255

CTG AAG GAG GAG GAG CTG GAA CGG AAG CCC AGC CTG TCA CTG ACG TTG 816
 Leu Lys Glu Glu Glu Leu Glu Arg Lys Pro Ser Leu Ser Leu Thr Leu
 260 265 270

ACC CTG GGG CGA GGC TG ACCACAACCA CTTATGGGAT ACCCGCACTC 863
 Thr Leu Gly Arg Gly
 275

TTCTTCCTGA GGCCGGACCC CGCCCAGGCA GGGAGCTGCT GTGAGTCCAG CTGATGCCCA 923

CCCAGGTGGT CCCTCCAGCC TGGTTAGGCA CTGAGGGTTC TGGACGGGCT CCAGGAACCC 983

TGGGCTGATG GGAGCAGTGA GCGGTTCCGC TGCCCCCTGC CCTGCACTGG ACCAGGAGTC 1043

TGGAGATGCC TGGGTAGCCC TCAGCATTG GAGGGGAACC TGTTCCCGTC GGTCCCCAAA 1103

TATCCCCTTC TTTTATGGG GTTAAGGAAG GGACCGAGAG ATCAGATAGT TGCTGTTTTG 1163

TAAAATGTAA TGTATATGTG GTTTTATAGTA AAATAGG3CA CCTGTTTCAC AAAAAAAAAA 1223

AAAAAAAAAA 1232

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 276 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Gln Arg Arg Trp Val Phe Val Leu Leu Asp Val Leu Cys Leu Leu
 1 5 10

Val Ala Ser Leu Pro Phe Ala Ile Leu Thr Leu Val Asn Ala Pro Tyr
 20 25 30

Lys Arg Gly Phe Tyr Cys Gly Asp Asp Ser Ile Arg Tyr Pro Tyr Arg
 35 40 45

Pro Asp Thr Ile Thr His Gly Leu Met Ala Gly Val Thr Ile Thr Ala
 50 55 60

Thr Val Ile Leu Val Ser Ala Gly Glu Ala Tyr Leu Val Tyr Thr Asp
 65 70 75 80
 Arg Leu Tyr Ser Arg Ser Asp Phe Asn Asn Tyr Val Ala Ala Val Tyr
 85 90 95
 Lys Val Leu Gly Thr Phe Leu Phe Gly Ala Ala Val Ser Gln Ser Leu
 100 105 110
 Thr Asp Leu Ala Lys Tyr Met Ile Gly Arg Leu Lys Pro Asn Phe Leu
 115 120 125
 Ala Val Cys Asp Pro Asp Trp Ser Arg Val Asn Cys Ser Val Tyr Val
 130 135 140
 Gln Leu Glu Lys Val Cys Arg Gly Asn Pro Ala Asp Val Thr Glu Ala
 145 150 155 160
 Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Gly Met Tyr Cys Met
 165 170 175
 Val Phe Leu Ala Leu Tyr Val Gln Ala Arg Leu Cys Trp Lys Trp Ala
 180 185 190
 Arg Leu Leu Arg Pro Thr Val Gln Phe Phe Leu Val Ala Phe Ala Leu
 195 200 205
 Tyr Val Gly Tyr Thr Arg Val Ser Asp Tyr Lys His His Trp Ser Asp
 210 215 220
 Val Leu Val Gly Leu Leu Gln Gly Ala Leu Val Ala Ala Leu Thr Val
 225 230 235 240
 Cys Tyr Ile Ser Asp Phe Phe Lys Ala Arg Pro Pro Gln His Cys Leu
 245 250 255
 Lys Glu Glu Glu Leu Glu Arg Lys Pro Ser Leu Ser Leu Thr Leu Thr
 260 265 270
 Leu Gly Arg Gly
 275

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 283 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Phe Asp Lys Thr Arg Leu Pro Tyr Val Ala Leu Asp Val Ile Cys
 1 5 10 15
 Val Leu Leu Ala Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr
 20 25 30
 Pro Phe Gln Arg Gly Ile Phe Cys Asn Asp Asp Ser Ile Lys Tyr Pro
 35 40 45
 Tyr Lys Glu Asp Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Val Ile
 50 55 60

Pro Phe Cys Ile Ile Val Met Ser Ile Gly Glu Ser Leu Ser Val Tyr
 65 70 75 80
 Phe Asn Val Leu His Ser Asn Ser Phe Val Gly Asn Pro Tyr Ile Ala
 85 90 95
 Thr Ile Tyr Lys Ala Val Gly Ala Phe Leu Phe Gly Val Ser Ala Ser
 100 105 110
 Gln Ser Leu Thr Asp Ile Ala Lys Tyr Thr Ile Gly Ser Leu Arg Pro
 115 120 125
 His Phe Leu Ala Ile Cys Asn Pro Asp Trp Ser Lys Ile Asn Cys Ser
 130 135 140
 Asp Gly Tyr Ile Glu Asp Tyr Ile Cys Gln Gly Asn Glu Glu Lys Val
 145 150 155 160
 Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser Met
 165 170 175
 Tyr Cys Met Leu Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys Gly
 180 185 190
 Asp Trp Ala Arg Leu Leu Arg Pro Met Leu Gln Phe Gly Leu Ile Ala
 195 200 205
 Phe Ser Ile Tyr Val Gly Leu Ser Arg Val Ser Asp Tyr Lys His His
 210 215 220
 Trp Ser Asp Val Thr Val Gly Leu Ile Gln Gly Ala Ala Met Ala Ile
 225 230 235 240
 Leu Val Ala Leu Tyr Val Ser Asp Phe Phe Lys Asp Thr His Ser Tyr
 245 250 255
 Lys Glu Arg Lys Glu Glu Asp Pro His Thr Thr Leu His Glu Thr Ala
 260 265 270
 Ser Ser Arg Asn Tyr Ser Thr Asn His Glu Pro
 275 280

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 284 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Phe Asp Lys Thr Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys
 1 5 10 15
 Val Leu Leu Ala Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr
 20 25 30
 Pro Phe Gln Arg Gly Val Phe Cys Asn Asp Glu Ser Ile Lys Tyr Pro
 35 40 45
 Tyr Lys Glu Asp Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Ile Ile
 50 55 60

Pro Phe Ser Ile Ile Val Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr
 65 70 75 80
 Cys Asn Leu Leu His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile Ala
 85 90 95
 Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala Ala Ser
 100 105 110
 Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg Leu Arg Pro
 115 120 125
 His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser Lys Ile Asn Cys Ser
 130 135 140
 Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg Gly Asn Ala Glu Arg Val
 145 150 155 160
 Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser Met
 165 170 175
 Tyr Cys Met Leu Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys Gly
 180 185 190
 Asp Trp Ala Arg Leu Leu Arg Pro Thr Leu Gln Phe Gly Leu Val Ala
 195 200 205
 Val Ser Ile Tyr Val Gly Leu Ser Arg Val Ser Asp Tyr Lys His His
 210 215 220
 Trp Ser Asp Val Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile
 225 230 235 240
 Leu Val Ala Val Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe
 245 250 255
 Lys Glu Arg Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro
 260 265 270
 Thr Thr Gly Asn His Tyr Pro Ser Asn His Gln Pro
 275 280

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 285 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Phe Asp Lys Thr Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys
 1 5 10
 Val Leu Leu Ala Ser Met Pro Met Ala Val Leu Lys Leu Gly Gln Ile
 20 25 30
 Tyr Pro Phe Gln Arg Gly Phe Phe Cys Lys Asp Asn Ser Ile Asn Tyr
 35 40 45
 Pro Tyr His Asp Ser Thr Ala Ala Ser Thr Val Leu Ile Leu Val Gly
 50 55 60

Val Gly Leu Pro Val Ser Ser Ile Ile Leu Gly Glu Thr Leu Ser Val
 65 70 75 80
 Tyr Cys Asn Leu Leu His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile
 85 90 95
 Ala Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala Ala
 100 105 110
 Ser Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg Leu Arg
 115 120 125
 Pro His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser Lys Ile Asn Cys
 130 135 140
 Ser Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg Gly Asn Ala Glu Arg
 145 150 155 160
 Val Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Ser
 165 170 175
 Met Tyr Cys Met Leu Phe Val Ala Leu Tyr Leu Gln Ala Arg Met Lys
 180 185 190
 Gly Asp Trp Ala Arg Leu Leu Arg Pro Thr Leu Gln Phe Gly Leu Val
 195 200 205
 Ala Val Ser Ile Tyr Val Gly Leu Ser Arg Val Ser Asp Tyr Lys His
 210 215 220
 His Trp Ser Asp Val Leu Thr Gly Leu Ile Gln Gly Ala Leu Val Ala
 225 230 235 240
 Ile Leu Val Ala Val Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser
 245 250 255
 Phe Lys Glu Arg Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr
 260 265 270
 Pro Thr Thr Gly Asn His Tyr Pro Ser Asn His Gln Pro
 275 280 285

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 311 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Gln Asn Tyr Lys Tyr Asp Lys Ala Ile Val Pro Glu Ser Lys Asn
 1 5 10 15
 Gly Gly Ser Pro Ala Leu Asn Asn Asn Pro Arg Arg Ser Gly Ser Lys
 20 25 30
 Arg Val Leu Leu Ile Cys Leu Asp Leu Phe Cys Leu Phe Met Ala Gly
 35 40 45
 Leu Pro Phe Leu Ile Ile Glu Thr Ser Thr Ile Lys Pro Tyr His Arg
 50 55 60

Gly Phe Tyr Cys Asn Asp Glu Ser Ile Lys Tyr Pro Leu Lys Thr Gly
 65 70 75 80
 Glu Thr Ile Asn Asp Ala Val Leu Cys Ala Val Gly Ile Val Ile Ala
 85 90 95
 Ile Leu Ala Ile Ile Thr Gly Glu Phe Tyr Arg Ile Tyr Tyr Leu Lys
 100 105 110
 Lys Ser Arg Ser Thr Ile Gln Asn Pro Tyr Val Ala Ala Leu Tyr Lys
 115 120 125
 Gln Val Gly Cys Phe Leu Phe Gly Cys Ala Ile Ser Gln Ser Phe Thr
 130 135 140
 Asp Ile Ala Lys Val Ser Ile Gly Arg Leu Arg Pro His Phe Leu Ser
 145 150 155 160
 Val Cys Asn Pro Asp Phe Ser Gln Ile Asn Cys Ser Glu Gly Tyr Ile
 165 170 175
 Gln Asn Tyr Arg Cys Arg Gly Asp Asp Ser Lys Val Gln Glu Ala Arg
 180 185 190
 Lys Ser Phe Phe Ser Gly His Ala Ser Phe Ser Met Tyr Thr Met Leu
 195 200 205
 Tyr Leu Val Leu Tyr Leu Gln Ala Arg Phe Thr Trp Arg Gly Ala Arg
 210 215 220
 Leu Leu Arg Pro Leu Leu Gln Phe Thr Leu Ile Met Met Ala Phe Tyr
 225 230 235 240
 Thr Gly Leu Ser Arg Val Ser Asp His Lys His His Pro Ser Asp Val
 245 250 255
 Leu Ala Gly Phe Ala Gln Gly Ala Leu Val Ala Cys Cys Ile Val Phe
 260 265 270
 Phe Val Ser Asp Leu Phe Lys Thr Lys Thr Thr Leu Ser Leu Pro Ala
 275 280 285
 Pro Ala Ile Arg Lys Glu Ile Leu Ser Pro Val Asp Ile Ile Asp Arg
 290 295 300
 Asn Asn His His Asn Met Met
 305 310

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 276 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Gln Arg Arg Trp Val Phe Val Leu Leu Asp Val Leu Cys Leu Leu
 1 5 10 15
 Val Ala Ser Leu Pro Phe Ala Ile Leu Thr Leu Val Asn Ala Pro Tyr
 20 25 30

Lys Arg Gly Phe Tyr Cys Gly Asp Asp Ser Ile Arg Tyr Pro Tyr Arg
 35 40 45
 Pro Asp Thr Ile Thr His Gly Leu Met Ala Gly Val Thr Ile Thr Ala
 50 55 60
 Thr Val Ile Leu Val Ser Ala Gly Glu Ala Tyr Leu Val Tyr Thr Asp
 65 70 75 80
 Arg Leu Tyr Ser Arg Ser Asp Phe Asn Asn Tyr Val Ala Ala Val Tyr
 85 90 95
 Lys Val Leu Gly Thr Phe Leu Phe Gly Ala Ala Val Ser Gln Ser Leu
 100 105 110
 Thr Asp Leu Ala Lys Tyr Met Ile Gly Arg Leu Lys Pro Asn Phe Leu
 115 120 125
 Ala Val Cys Asp Pro Asp Trp Ser Arg Val Asn Cys Ser Val Tyr Val
 130 135 140
 Gln Leu Glu Lys Val Cys Arg Gly Asn Pro Ala Asp Val Thr Glu Ala
 145 150 155 160
 Arg Leu Ser Phe Tyr Ser Gly His Ser Ser Phe Gly Met Tyr Cys Met
 165 170 175
 Val Phe Leu Ala Leu Tyr Val Gln Ala Arg Leu Cys Trp Lys Trp Ala
 180 185 190
 Arg Leu Leu Arg Pro Thr Val Gln Phe Phe Leu Val Ala Phe Ala Leu
 195 200 205
 Tyr Val Gly Tyr Thr Arg Val Ser Asp Tyr Lys His His Trp Ser Asp
 210 215 220
 Val Leu Val Gly Leu Leu Gln Gly Ala Leu Val Ala Ala Leu Thr Val
 225 230 235 240
 Cys Tyr Ile Ser Asp Phe Phe Lys Ala Arg Pro Pro Gln His Cys Leu
 245 250 255
 Lys Glu Glu Glu Leu Glu Arg Lys Pro Ser Leu Ser Leu Thr Leu Thr
 260 265 270
 Leu Gly Arg Gly
 275

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGCTCTAGAT ATTAATAGTA ATCAATTAC

29

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTCACGCGAT GCACCATGGT AATAGC

26

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGCATGCG TGAGGCTCCG GTGC

24

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTAGTTTTCA CGGTACCTGA AATGGAAG

28

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGCATGGTAC CATGTTTGAC AAGACGCGGC

30

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CATATGTAGT ATTCAATGTA ACC

23

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 47 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGATGGCTAG CATGCAGAGA AGATGGGTCT TCGTGCTGCT CGACGTG

47

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGTGCGGGAT CCCATAAGTG GTTG

24

What Is Claimed Is:

1. An isolated polynucleotide encoding human phosphatidic acid phosphatase wherein said polynucleotide encodes a protein comprising a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1 (SEQ ID NO:2), (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2 (SEQ ID NO:4), and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4 (SEQ ID NO:8).

2. An isolated human phosphatidic acid phosphatase protein, wherein said protein comprises a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1 (SEQ ID NO:2), (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2 (SEQ ID NO:4), and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4 (SEQ ID NO:8).

3. A method of preparing a human phosphatidic acid phosphatase- β protein comprising the steps of (i) transforming a host cell with an expression vector comprising a polynucleotide encoding human phosphatidic acid phosphatase, (ii) culturing said transformed host cells which express said protein and (iii) isolating said protein.

4. The method of claim 3, wherein said polynucleotide encoding human phosphatidic acid is selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1 (SEQ ID NO:2), (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2 (SEQ ID NO:4), (iii) the sequence at amino acid number 1 to amino acid number 311 in Figure 3 (SEQ ID NO:6), and (iv) the sequence at

amino acid number 1 to amino acid number 276 in Figure 4 (SEQ ID NO:8).

5 5. A method of dephosphorylating a substrate comprising recombinantly producing a human phosphatidic acid phosphatase protein and contacting said substrate with an effective amount of said recombinantly produced human phosphatidic acid phosphatase protein such that
10 said protein catalyzes the dephosphorylation of said substrate.

15 6. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 284 in Figure 1 (SEQ ID NO:2).

 7. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 285 in Figure 2 (SEQ ID NO:4).

20 8. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 311 in Figure 3 (SEQ ID NO:6).

25 9. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 276 in Figure 4 (SEQ ID NO:8).

30 10. The method of claim 5, wherein said substrate is selected from the group consisting of phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate.

35 11. The method of claim 5, wherein said contacting is effected *in vitro*, and further comprises the step of isolating said dephosphorylated substrate.

12. The method of claim 5, wherein said contacting step occurs in vivo and is effected by the administration of said human phosphatidic acid phosphatase to a mammal in need thereof.

5

13. A method of dephosphorylating a substrate comprising contacting said substrate with an effective amount of isolated human phosphatidic acid phosphatase protein such that said protein catalyzes the dephosphorylation of said substrate, wherein said substrate is selected from the group consisting of lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate.

10

Fig. 1A

CCTGTGGGAGAGAGCGCCGGGATCCGGACGGGGTAGCAACCGGGGAGGCCGTGCCGGCTGA	62
GGAGGTCCTGAGGCTACAGAGCTGCCGCGGCTGGCACACGAGCGCCTCGGCACTAACC	122
GTGTTTCGCGGGGGCTGTGAGGGGAGGGCCCCGGGCGCCATTGCTGGCGGTGGGAGCGCCG	182
CCCGGTCTCAGCCCCGCCCTCGGCTGCTCTCCTCCTCCGGCTGGGAGGGGCGGTATCTCGG	242
GGCCGTCGCCAGCCCCGGCCCGGGCTCGATAATCAAGGGCCTCGGCCGTCGTCCCGCACC	302
TCATTCCATCGCCCTTGCCGGGAGCCCCGGGAGAGACC	356
Met Phe Asp Lys Thr	5
CGG CTG CCG TAC GTG GCC CTC GAT GTG CTC TGC GTG TTG CTG GCT	401
Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys Val Leu Leu Ala	10
GGA TTG CCT TTT GCA ATT CTT ACT TCA AGG CAT ACC CCC TTC CAA	20
Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr Pro Phe Gln	25
CGA GGA GTA TTC TGT AAT GAT GAG TCC ATC AAG TAC CCT TAC AAA	30
Arg Gly Val Phe Cys Asn Asp Glu Ser Ile Lys Tyr Pro Tyr Lys	35
GAA GAC ACC ATA CCT TAT GCG TTA TTA GGT GGA ATA ATC ATT CCA	40
Glu Asp Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Ile Ile Pro	45
TTC AGT ATT ATC GTT ATT ATT CTT GGA GAA ACC CTG TCT GTT TAC	50
Phe Ser Ile Ile Val Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr	55
TGT AAC CTT TTG CAC TCA AAT TCC TTT ATC AGG AAT AAC TAC ATA	60
Cys Asn Leu Leu His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile	65
GCC ACT ATT TAC AAA GCC ATT GGA ACC TTT TTA TTT GGT GCA GCT	70
Ala Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala	75
GCT AGT CAG TCC CTG ACT GAC ATT GCC AAG TAT TCA ATA GGC AGA	80
Ala Ser Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg	85
CTG CGG CCT CAC TTC TTG GAT GTT TGT GAT CCA GAT TGG TCA AAA	90
Leu Arg Pro His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser Lys	95
ATC AAC TGC AGC GAT GGT TAC ATT GAA TAC TAC ATA TGT CGA GGG	100
Ile Asn Cys Ser Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg Gly	105
AAT GCA GAA AGA GTT AAG GAA GGC AGG TTG TCC TTC TAT TCA GGC	110
Asn Ala Glu Arg Val Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly	115
CAC TCT TCG TTT TCC ATG TAC TGC ATG CTG TTT GTG GCA CTT TAT	120
His Ser Ser Phe Ser Met Tyr Cys Met Leu Phe Val Ala Leu Tyr	125
CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA AGA CTC TTA CGC CCC	130
Leu Gln Ala Arg Met Lys Gly Asp Trp Ala Arg Leu Leu Arg Pro	135
ACA CTG CAA TTT GGT CTT GTT GCC GTA TCC ATT TAT GTG GGC CTT	140
Thr Leu Gln Phe Gly Leu Val Ala Val Ser Ile Tyr Val Gly Leu	145
TCT CGA GTT TCT GAT TAT AAA CAC CAC TGG AGC GAT GTG TTG ACT	150
Ser Arg Val Ser Asp Tyr Lys His His Trp Ser Asp Val Leu Thr	155
GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT GTA TAT	160
Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala Val Tyr	165
GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA AAA	170
Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg Lys	175
	180
	185
	190
	195
	200
	205
	210
	215
	220
	225
	230
	235
	240
	245
	250
	255
	260

Fig. 1B

GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT GGG	1166
Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr Gly	
265 270 275	
AAT CAC TAT CCG AGC AAT CAC CAG CCT TGA AAG GCAGCAGGGTGCCCAG	1215
Asn His Tyr Pro Ser Asn His Gln Pro ***	
280	
GTGAAGCTGGCCTGTTTTCTAAAGGAAAATGATTGCCACAAGGCAAGAGGATGCATCTTT	1275
CTTCCTGGTGTACAAGCCTTTAAAGACTTCTGCTGCTGATATGCCTCTTGGATGCACACT	1335
TTGTGTGTACATAGTTACCTTTAACTCAGTGGTTATCTAATAGCTCTAACTCATTAATA	1395
AAACTCCAAGCCTTCCACCAAAACAGTGCCCCACCTGTATACATTTTTATTAAAAAATG	1455
TAATGCTTATGTATAAACATGTATGTAATATGCTTTCTATGAATGATGTTTGATTAAAT	1515
ATAATACATATTAAATGTATGGGAGAACCAAAAAAAAAAAAAAAAAAAAA	1563

Fig. 2A

CCTGTGGGAGAGAGCGCCGGGATCCGGACGGGGTAGCAACCGGGGCAGGCCGTGCCGGCTGA	62
GGAGGTCTGAGGCTACAGAGCTGCCCGGGCTGGCACACGAGCGCCTCGGCACTAACCGA	122
GTGTTTCGGGGGGCTGTGAGGGGAGGGCCCCGGGCGCCATTGCTGGCGGTGGGAGCGCCG	182
CCCGGTCTCAGCCCCGCCCTCGGCTGCTCTCCTCCTCCGGCTGGGAGGGGCGGTATCTCGG	242
GGCCGTCGCCAGCCCCGGGCGGGCTCGATAATCAAGGGCCTCGGCCGTCGTCCCGCACC	302
TCATTCCATCGCCCTTGCCGGGCGAGCCCGGGCAGAGACC ATG TTT GAC AAG ACG	356
Met Phe Asp Lys Thr	5
CGG CTG CCG TAC GTG GCC CTC GAT GTG CTC TGC GTG TTG CTG GCT	401
Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys Val Leu Leu Ala	10 15 20
TCC ATG CCT ATG GCT GTT CTA AAA TTG GGC CAA ATA TAT CCA TTT	446
Ser Met Pro Met Ala Val Leu Lys Leu Gly Gln Ile Tyr Pro Phe	25 30 35
CAG AGA GGC TTT TTC TGT AAA GAC AAC AGC ATC AAC TAT CCG TAC	491
Gln Arg Gly Phe Phe Cys Lys Asp Asn Ser Ile Asn Tyr Pro Tyr	40 45 50
CAT GAC AGT ACC GCC GCA TCC ACT GTC CTC ATC CTA GTG GGG GTT	536
His Asp Ser Thr Ala Ala Ser Thr Val Leu Ile Leu Val Gly Val	55 60 65
GGC TTG CCC GTT TCC TCT ATT ATT CTT GGA GAA ACC CTG TCT GTT	581
Gly Leu Pro Val Ser Ser Ile Ile Leu Gly Glu Thr Leu Ser Val	70 75 80
TAC TGT AAC CTT TTG CAC TCA AAT TCC TTT ATC AGT AAT AAC TAC	626
Tyr Cys Asn Leu Leu His Ser Asn Ser Phe Ile Ser Asn Asn Tyr	85 90 95
ATA GCC ACT ATT TAC AAA GCC ATT GGA ACC TTT TTA TTT GGT GCA	671
Ile Ala Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala	100 105 110
GCT GCT AGT CAG TCC CTG ACT GAC ATT GCC AAG TAT TCA ATA GGC	716
Ala Ala Ser Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly	115 120 125
AGA CTG CGG CCT CAC TTC TTG GAT GTT TGT GAT CCA GAT TGG TCA	761
Arg Leu Arg Pro His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser	130 135 140
AAA ATC AAC TGC AGC GAT GGT TAC ATT GAA TAC TAC ATA TGT CGA	806
Lys Ile Asn Cys Ser Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg	145 150 155
GGG AAT GCA GAA AGA GTT AAG GAA GGC AGG TTG TCC TTC TAT TCA	851
Gly Asn Ala Glu Arg Val Lys Gly Gly Arg Leu Ser Phe Tyr Ser	160 165 170
GGC CAC TCT TCG TTT TCC ATG TAC TGC ATG CTG TTT GTG GCA CTT	896
Gly His Ser Ser Phe Ser Met Tyr Cys Met Leu Phe Val Ala Leu	175 180 185
TAT CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA AGA CTC TTA CGC	941
Tyr Leu Gln Ala Arg Met Lys Gly Asp Trp Ala Arg Leu Leu Arg	190 195 200
CCC ACA CTG CAA TTT GGT CTT GTT GCC GTA TCC ATT TAT GTG GGC	986
Pro Thr Leu Gln Phe Gly Leu Val Ala Val Ser Ile Tyr Val Gly	205 210 215
CTT TCT CGA GTT TCT GAT TAT AAA CAC CAC TGG AGC GAT GTG TTG	1031
Leu Ser Arg Val Ser Asp Tyr Lys His His Trp Ser Asp Val Leu	220 225 230
ACT GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT GTA	1076
Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala Val	235 240 245

Fig. 2B

TAT GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA	1121
Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg	
250 260	
AAA GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT	1166
Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr	
265 270 275	
GGG AAT CAC TAT CCG AGC AAT CAC CAG CCT TGA AAGGCAGCAGGGTGCC	1215
Gly Asn His Tyr Pro Ser Asn His Gln Pro ***	
280 285	
CAGGTGAAGCTGGCCTGTTTTCTAAAGGAAAATGATTGCCACAAGGCAAGAGGATGCATC	1275
TTTCTTCCTGGTGTACAAGCCTTTAAAGACTTCTGCTGCTGATATGCCTCTTGGATGCAC	1335
ACTTTGTGTGTACATAGTTACCTTTAACTCAGTGGTTATCTAATAGCTCTAAACTCATTA	1395
AAAAAACTCCAAGCCTTCCACCAAAACAGTGCCCCACCTGTATACATTTTATTAAAAAA	1455
ATGTAATGCTTATGTATAAACATGTATGTAATATGCTTTCTATGAATGATGTTTGATTTA	1515
AATATAATACATATTAAAATGTATGGGAGAACCAAAAAAAAAAAAAAAAAAAAAA	1566

Fig. 3A

GGCGCAGCTCTGCAAAAGTTTCTGCTCGGGATCTGGCTCTCTTCCCCTTGGACTTTAGAACG 62
ATTTAGGGTTGACAGAGGAAAGCAGAGGCGCGCAGGAGGAGCAGAAAACACCACCTTCTG 122
CAGTTGGAGGCAGGCAGCCCCGGCTGCACTCTAGCCGCCGCGCCGGAGCCGGGGCCGAC 182
CCGCCACTATCCGCAGCAGCCTCGGCCAGGAGGCGACCCGGGCGCCTGGGTGTGTGGCTG 242
CTGTTGCGGGACGTCTTCGCGGGGCGGGAGGCTCGCGCCGCAGCCAGCGCC ATG CAA 299
Met Gln
AAC TAC AAG TAC GAC AAA GCG ATC GTC CCG GAG AGC AAG AAC GGC 344
Asn Tyr Lys Tyr Asp Lys Ala Ile Val Pro Glu Ser Lys Asn Gly
5 10 15
GGC AGC CCG GCG CTC AAC AAC AAC CCG AGG AGG AGC GGC AGC AAG 389
Gly Ser Pro Ala Leu Asn Asn Asn Pro Arg Arg Ser Gly Ser Lys
20 25 30
CGG GTG CTG CTC ATC TGC CTC GAC CTC TTC TGC CTC TTC ATG GCG 434
Arg Val Leu Leu Ile Cys Leu Asp Leu Phe Cys Leu Phe Met Ala
35 40 45
GGC CTC CCC TTC CTC ATC ATC GAG ACA AGC ACC ATC AAG CCT TAC 479
Gly Leu Pro Phe Leu Ile Ile Glu Thr Ser Thr Ile Lys Pro Tyr
50 55 60
CAC CGA GGG TTT TAC TGC AAT GAT GAG AGC ATC AAG TAC CCA CTG 524
His Arg Gly Phe Tyr Cys Asn Asp Glu Ser Ile Lys Tyr Pro Leu
65 70 75
AAA ACT GGT GAG ACA ATA AAT GAC GCT GTG CTC TGT GCC GTG GGG 569
Lys Thr Gly Glu Thr Ile Asn Asp Ala Val Leu Cys Ala Val Gly
80 85 90
ATC GTC ATT GCC ATC CTC GCG ATC ATC ACG GGG GAA TTC TAC CGG 614
Ile Val Ile Ala Ile Leu Ala Ile Ile Thr Gly Glu Phe Tyr Arg
95 100 105
ATC TAT TAC CTG AAG AAG TCG CGG TCG ACG ATT CAG AAC CCC TAC 659
Ile Tyr Tyr Leu Lys Lys Ser Arg Ser Thr Ile Gln Asn Pro Tyr
110 115 120
GTG GCA GCA CTC TAT AAG CAA GTG GGC TGC TTC CTC TTT GGC TGT 704
Val Ala Ala Leu Tyr Lys Gln Val Gly Cys Phe Leu Phe Gly Cys
125 130 135
GCC ATC AGC CAG TCT TTC ACA GAG ATT GCC AAA GTG TCC ATA GGG 749
Ala Ile Ser Gln Ser Phe Thr Asp Ile Ala Lys Val Ser Ile Gly
140 135 150
CGC CTG CGT CCT CAC TTC TTG AGT GTC TGC AAC CCT GAT TTC AGC 794
Arg Leu Arg Pro His Phe Leu Ser Val Cys Asn Pro Asp Phe Ser
155 160 165
CAG ATC AAC TGC TCT GAA GGC TAC ATT CAG AAC TAC AGA TGC AGA 839
Gln Ile Asn Cys Ser Glu Gly Tyr Ile Gln Asn Tyr Arg Cys Arg
170 180
GGT GAT GAC AGC AAA GTC CAG GAA GCC AGG AAG TCC TTC TTC TCT 884
Gly Asp Asp Ser Lys Val Gln Glu Ala Arg Lys Ser Phe Phe Ser
185 190 195
GGC CAT GCC TCC TTC TCC ATG TAC ACT ATG CTG TAT TTG GTG CTA 929
Gly His Ala Ser Phe Ser Met Tyr Thr Met Leu Tyr Leu Val Leu
200 205 210
TAC CTG CAG GCC CGC TTC ACT TGG CGA GGA GCC CGC CTG CTC CGG 974
Tyr Leu Gln Ala Arg Phe Thr Trp Arg Gly Ala Arg Leu Leu Arg
215 220 225
CCC CTC CTG CAG TTC ACC TTG ATC ATG ATG GCC TTC TAC ACG GGA 1019
Pro Leu Leu Gln Phe Thr Leu Ile Met Met Ala Phe Tyr Thr Gly
230 235 240
CTG TCT CGC GTA TCA GAC CAC AAG CAC CAT CCC AGT GAT GTT CTG 1064
Leu Ser Arg Val Ser Asp His Lys His His Pro Ser Asp Val Leu
245 250 255

Fig. 3B

GCA GGA TTT GCT CAA GGA GCC CTG GTG GCC TGC TGC ATA GTT TTC	1109
Ala Gly Phe Ala Gln Gly Ala Leu Val Ala Cys Cys Ile Val Phe	
260 265 270	
TTC GTG TCT GAC CTC TTC AAG ACT AAG ACG ACG CTC TCC CTG CCT	1154
Phe Val Ser Asp Leu Phe Lys Thr Lys Thr Thr Leu Ser Leu Pro	
275 280 285	
GCC CCT GCT ATC CGG AAG GAA ATC CTT TCA CCT GTG GAC ATT ATT	1199
Ala Pro Ala Ile Arg Lys Glu Ile Leu Ser Pro Val Asp Ile Ile	
290 295 300	
GAC AGG AAC AAT CAC CAC AAC ATG ATG TAG GTGCCACCCACCTCCTGAGC	1249
Asp Arg Asn Asn His His Asn Met Met ***	
305 310	
TGTTTTTGTAAAATGACTGCTGACAGCAAGTTCTTGCTGCTCTCCAATCTCATCAGACAG	1309
TAGAATGTAGGGAAAACTTTTGCCCGACTGATTTTAAAAAAAAAAAAAAAAAAAA	1362

Fig. 4A

ACC	ATG	CAG	CGG	AGG	TGG	GTC	TTC	GTG	CTG	CTC	GAC	GTG	CTG	TGC	47
Met	Gln	Arg	Arg	Trp	Val	Phe	Val	Leu	Leu	Asp	Val	Leu	Cys		
				5						10					
TTA	CTG	GTC	GCC	TCC	CTG	CCC	TTC	GCT	ATC	CTG	ACG	CTG	GTG	AAC	92
Leu	Leu	Val	Ala	Ser	Leu	Pro	Phe	Ala	Ile	Leu	Thr	Leu	Val	Asn	
	15				20					25					
GCC	CCG	TAC	AAG	CGA	GGA	TTT	TAC	TGC	GGG	GAT	GAC	TCC	ATC	CGG	137
Ala	Pro	Tyr	Lys	Arg	Gly	Phe	Tyr	Cys	Gly	Asp	Asp	Ser	Ile	Arg	
	30				35					40					
TAC	CCC	TAC	CGT	CCA	GAT	ACC	ATC	ACC	CAC	GGG	CTC	ATG	GCT	GGG	182
Tyr	Pro	Tyr	Arg	Pro	Asp	Thr	Ile	Thr	His	Gly	Leu	Met	Ala	Gly	
	45				50					55					
GTC	ACC	ATC	ACG	GCC	ACC	GTC	ATC	CTT	GTC	TCG	GCC	GGG	GAA	GCC	227
Val	Thr	Ile	Thr	Ala	Thr	Val	Ile	Leu	Val	Ser	Ala	Gly	Glu	Ala	
	60				65					70					
TAC	CTG	GTG	TAC	ACA	GAC	CGG	CTC	TAT	TCT	CGC	TCG	GAC	TTC	AAC	272
Tyr	Leu	Val	Tyr	Thr	Asp	Arg	Leu	Tyr	Ser	Arg	Ser	Asp	Phe	Asn	
	75				80					85					
AAC	TAC	GTG	GCT	GCT	GTA	TAC	AAG	GTG	CTG	GGG	ACC	TTC	CTG	TTT	317
Asn	Tyr	Val	Ala	Ala	Val	Tyr	Lys	Val	Leu	Gly	Thr	Phe	Leu	Phe	
	90				95					100					
GGG	GCT	GCC	GTG	AGC	CAG	TCT	CTG	ACA	GAC	CTG	GCC	AAG	TAC	ATG	362
Gly	Ala	Ala	Val	Ser	Gln	Ser	Leu	Thr	Asp	Leu	Ala	Lys	Tyr	Met	
	105				110					115					
ATT	GGG	CGT	CTG	AAG	CCC	AAC	TTC	CTA	GCC	GTC	TGC	GAC	CCC	GAC	407
Ile	Gly	Arg	Leu	Lys	Pro	Asn	Phe	Leu	Ala	Val	Cys	Asp	Pro	Asp	
	120				125					130					
TGG	AGC	CGG	GTC	AAC	TGC	TCG	GTC	TAT	GTG	CAG	CTG	GAG	AAG	GTG	452
Trp	Ser	Arg	Val	Asn	Cys	Ser	Val	Tyr	Val	Gln	Leu	Glu	Lys	Val	
	135				140					145					
TGC	AGG	GGA	AAC	CCT	GCT	GAT	GTC	ACC	GAG	GCC	AGG	TTG	TCT	TTC	497
Cys	Arg	Gly	Asn	Pro	Ala	Asp	Val	Thr	Glu	Ala	Arg	Leu	Ser	Phe	
	150				155					160					
TAC	TCG	GGA	CAC	TCT	TCC	TTT	GGG	ATG	TAC	TGC	ATG	GTG	TTC	TTG	542
Tyr	Ser	Gly	His	Ser	Ser	Phe	Gly	Met	Tyr	Cys	Met	Val	Phe	Leu	
	165				170					175					
GCG	CTG	TAT	GTG	CAG	GCA	CGA	CTC	TGT	TGG	AAG	TGG	GCA	CGG	CTG	587
Ala	Leu	Tyr	Val	Gln	Ala	Arg	Leu	Cys	Trp	Lys	Trp	Ala	Arg	Leu	
	180				185					190					
CTG	CGA	CCC	ACA	GTC	CAG	TTC	TTC	CTG	GTG	GCC	TTT	GCC	CTC	TAC	632
Leu	Arg	Pro	Thr	Val	Gln	Phe	Phe	Leu	Val	Ala	Phe	Ala	Leu	Tyr	
	195				200					205					
GTG	GGC	TAC	ACC	CGC	GTG	TCT	GAT	TAC	AAA	CAC	CAC	TGG	AGC	GAT	677
Val	Gly	Tyr	Thr	Arg	Val	Ser	Asp	Tyr	Lys	His	His	Trp	Ser	Asp	
	210				215					220					
GTC	CTT	GTT	GGC	CTC	CTG	CAG	GGG	GCA	CTG	GTG	GCT	GCC	CTC	ACT	722
Val	Leu	Val	Gly	Leu	Gln	Gly	Ala	Leu	Val	Ala	Ala	Leu	Thr		
	225				230					235					
GTC	TGC	TAC	ATC	TCA	GAC	TTC	TTC	AAA	GCC	CGA	CCC	CCA	CAG	CAC	767
Val	Cys	Tyr	Ile	Ser	Asp	Phe	Phe	Lys	Ala	Arg	Pro	Pro	Gln	His	
	240				245					250					
TGT	CTG	AAG	GAG	GAG	GAG	CTG	GAA	CGG	AAG	CCC	AGC	CTG	TCA	CTG	812
Cys	Leu	Lys	Glu	Glu	Glu	Leu	Glu	Arg	Lys	Pro	Ser	Leu	Ser	Leu	
	255				260					265					
ACG	TTG	ACC	CTG	GGG	CGA	GGC	TGA	CCACA	ACCACTTATGGG	GATACCCGCACT					864
Thr	Leu	Thr	Leu	Gly	Arg	Gly	***								
	270				275										

Fig. 4B

CTTCTTCCTGAGGCCGGACCCCGCCCAGGCAGGGAGCTGCTGTGAGTCCAGCTGATGCCC	924
ACCCAGGTGGTCCCTCCAGCCTGGTTAGGCACTGAGGGTTCTGGACGGGCTCCAGGAACC	984
CTGGGCTGATGGGAGCAGTGAGCGGTTCCGCTGCCCCCTGCCCTGCACTGGACCAGGAGT	1044
CTGGAGATGCCTGGGTAGCCCTCAGCATTTGGAGGGGAACCTGTTCCCGTCGGTCCCCAA	1104
ATATCCCCTTCTTTTTATGGGGTTAAGGAAGGGACCGAGAGATCAGATAGTTGCTGTTTT	1164
GTAAATGTAATGTATATGTGGTTTTTAGTAAATAGGGCACCTGTTTCACAAAAAAAAAA	1224
AAAAAAAAA	1234

Fig. 5

	10	20	30	40	50
M_PAP.AMI	1				
PAP_A1.AMI	1				
PAP_A2.AMI	1				
PAP_B.AMI	1	NYKYDRAI	VPESKNGGSP	ALNNNPRRSG	SKGVLLIC
PAP_G.AMI	1				
	60	70	80	90	100
M_PAP.AMI	51				
PAP_A1.AMI	51				
PAP_A2.AMI	51	MVVKLGQY	VGKQ	NSNMPHDS	HAASTIL
PAP_B.AMI	51	LIESTK	HLTG	ENDYCA	VIA LA
PAP_G.AMI	51	LVNA	KGGG	THGMA	TATLV
	110	120	130	140	150
M_PAP.AMI	101	SISFS	FV	G	CALVS
PAP_A1.AMI	101				
PAP_A2.AMI	101				
PAP_B.AMI	101	TFYRIY	KKSRST	QV	IECF
PAP_G.AMI	101	AYLT	DRYSRDE	VLE	VGG
	160	170	180	190	200
M_PAP.AMI	151				
PAP_A1.AMI	151				
PAP_A2.AMI	151				
PAP_B.AMI	151				
PAP_G.AMI	151				
	210	220	230	240	250
M_PAP.AMI	201				
PAP_A1.AMI	201				
PAP_A2.AMI	201				
PAP_B.AMI	201				
PAP_G.AMI	201				
	260	270	280	290	300
M_PAP.AMI	251				
PAP_A1.AMI	251				
PAP_A2.AMI	251				
PAP_B.AMI	251				
PAP_G.AMI	251				
	310	320	330	340	350
M_PAP.AMI	301	ASSRN	ES	TNE*	
PAP_A1.AMI	301				
PAP_A2.AMI	301				
PAP_B.AMI	301	PVDIIDRNNH	HMM*		
PAP_G.AMI	301	RKLSLTLT	LGRG*		

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Fig. 6

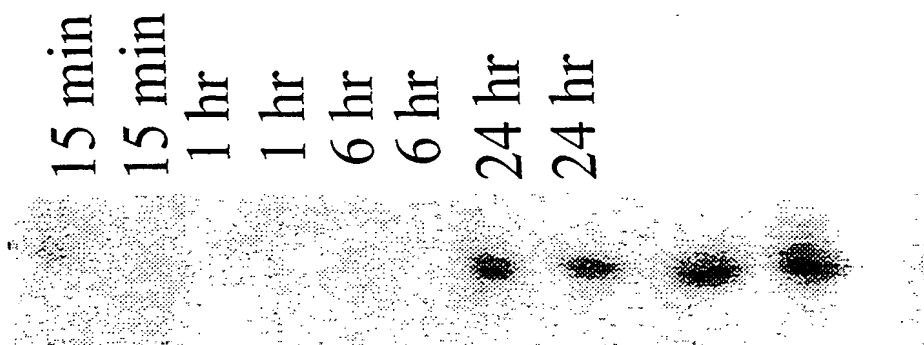
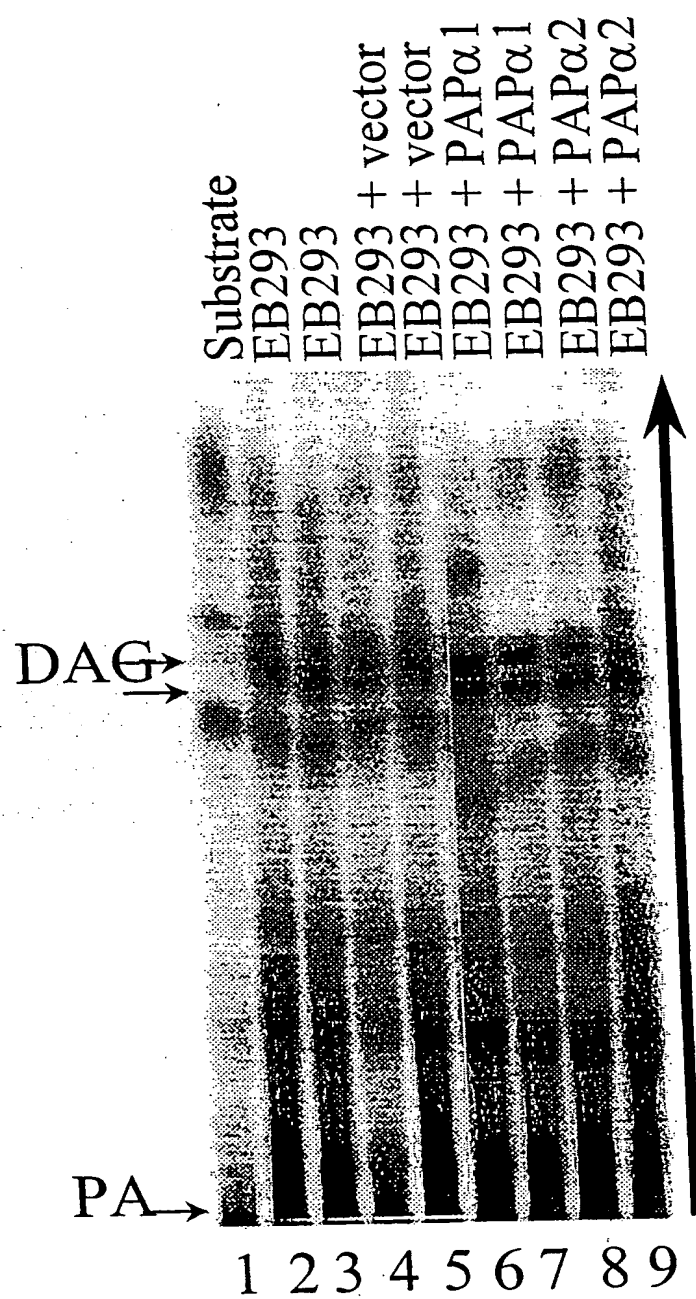


Fig. 7



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Fig. 8

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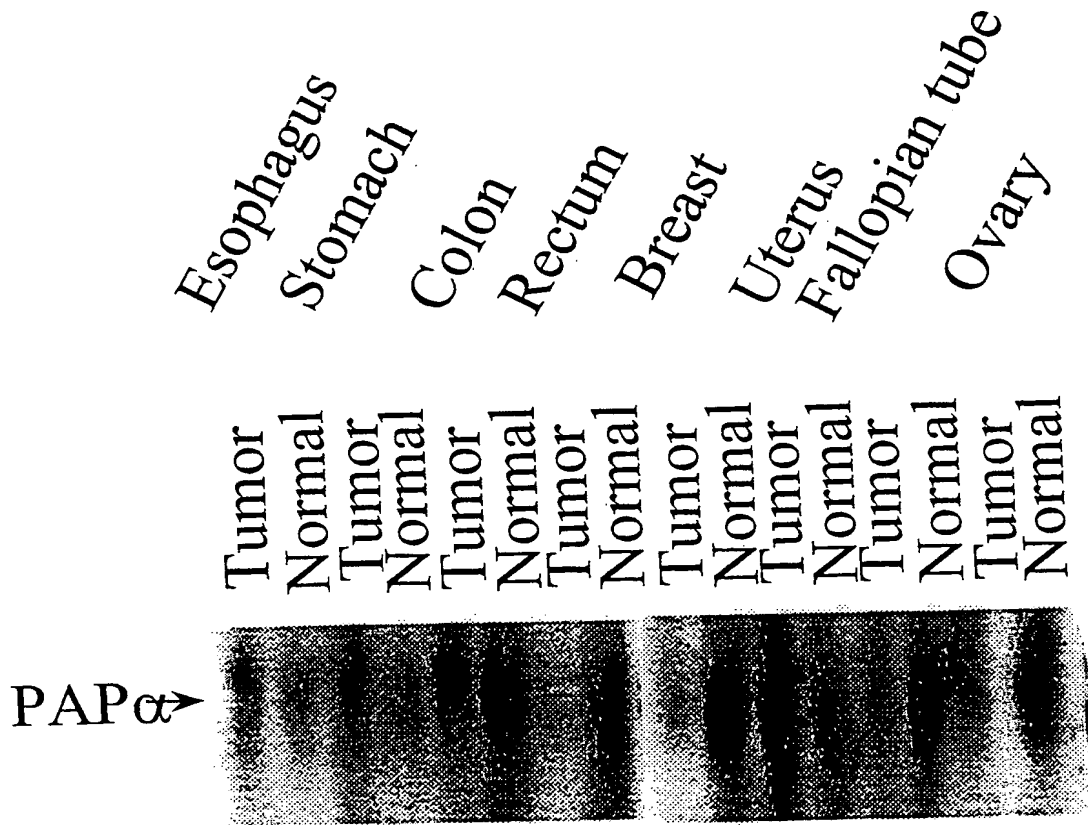
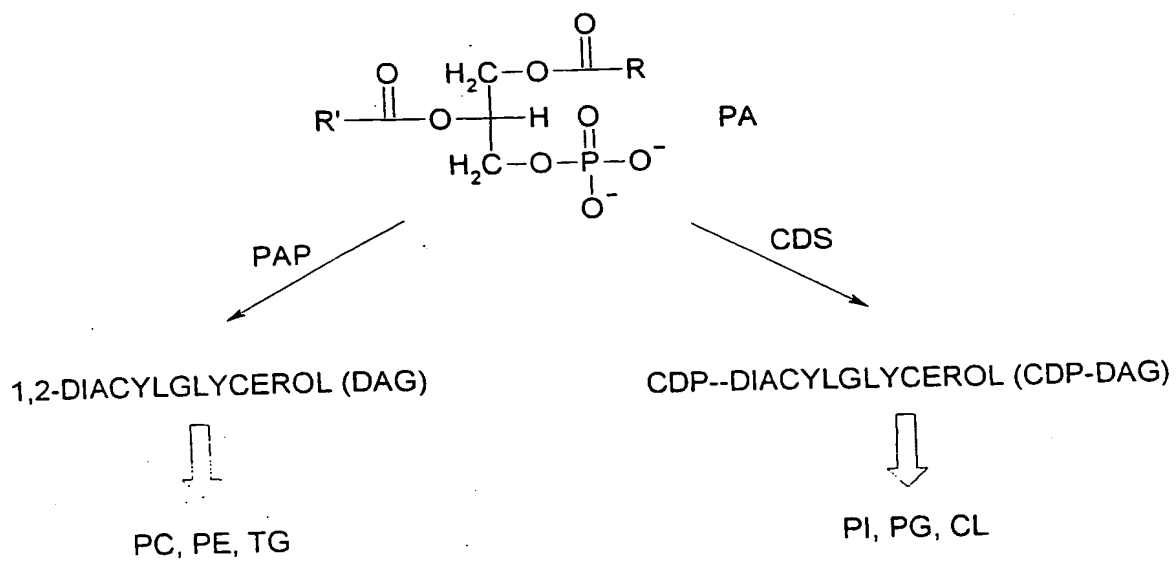


Fig. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07928

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 9/16, 15/55; C12P 13/02, 7/64, 7/62

US CL :536/23.2; 435/196, 128, 134, 135, 147

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 435/196, 128, 134, 135, 147

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAI, M. et al. Identification and cDNA Cloning of 35-kDa Phosphatidic Acid Phosphatase (Type 2) Bound to Plasma Membranes. The Journal of Biological Chemistry, 02 August 1996. Vol. 271, No. 31, pages 18931-18938. see entire document.	2, 3, 5, 6, 10-13
Y	Database GENBANK on STN, National Institute of Health, (Bethesda MD), Accession No. AA040858, HILLIER et al., The WashU-Merck EST Project, 30 August 1996.	2, 3, 5, 6, 10-13
Y	Database GENBANK on STN, National Institute of Health (Bethesda MD), Accession No. H68363, HILLIER et al., The WashU-Merck EST Project, 18 October 1995.	2, 3, 5, 6, 10-13



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database GENBANK on STN, National Institute of Health (Bethesda MD), Accession No. W04968, HILLIER et al., The WashU-Merck EST Project, 23 April 1996.	2, 3, 5, 6, 10-13
Y	Database GENBANK on STN, National Institute of Health, (Bethesda MD), Accession No. U79294, YU et al., Large Scale Concatenation cDNA Sequencing, 25 March 1997.	3, 4
Y	BRINDLEY, D.N. Phosphatidate Phosphohydrolase and Signal Transduction. Chemistry and Physics of Lipids. 1996. Vol. 80, pages 45-57, especially pages 47-50.	13

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International application No.

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B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, CAPLUS, NTIS, WPI
search terms: phosphatidic acid or phosphatidate, phosphatase# or phosphohydrolase#, human or isolat? or purif? or
gene# or sequence#

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